06

IMPORTANT NORMAL STANDARDS.

(For general considerations see page 120).

Hemoglobin: 15.6 gm. = 100 per cent.

Adult Males: Average 15.6 gm. (100 per cent) (13.5 to 19.5 or 85 per cent to 120 per cent).

Adult Females: Average 14.3 gm. (92 per cent) (11 to 16.5 or 72 per cent to 105 per cent).

Children six months to two years: Average 12.5 gm. (80 per cent) (9.4 to 15.4 or 60 to 98 per cent).

Infants one day to two weeks: Average 20 gm. (128 per cent) (15.5 to 26.3 or 99 per cent to 168 per cent).

Red Cells, Millions in a Cubic Millimeter:

Adult males average 5.4 (4.4 to 6.4).

Adult females average 4.8 (4.07 to 5.55).

Children (one to two years) (5 to 6).

Infants average 7.5 (6 to 9).

Leucocytes, Total in a Cubic Millimeter 4,000 to 12,000.

Differential percentage:

Lymphocytes: 20 to 30 per cent.

Monocytes: 5 to 10 per cent.

Eosinophiles: 2 to 5 per cent.

Neutrophiles: 60 to 75 per cent (unsegmented 5 to 10, segmented 55 to 70).

Myelocytes: less than 1 per cent.

Infants and young children have 50 per cent more lymphocytes.

Blood Platelets: 200,000 to 400,000 per cubic millimeter.

Bleeding time two to five minutes.

Clotting time two to eight minutes.

Red Cell Fragility:

Beginning hemolysis 0.45 NaCl.

Complete hemolysis 0.35 NaCl.

Cell Diameter: 7.5 microns (6 to 9).

Corpuscular Volume: Average 45 per cent (36 per cent to 52 per cent).

Cell sedimentation 2 to 15 mm. per hour, average of 6.

Total Quantity: plasma—5 per cent of body weight.

Total blood 8.8 per cent (7.8 to 9.7) of body weight.

IMPORTANT NORMAL STANDARDS

Blood Chemical.—(The following constituents are in milligrams per 100 cc. unless otherwise stated):

Urea 20 to 40.
Urea nitrogen 10 to 20.
Creatinine 1 to 2.
Uric acid 2 to 4.
Sugar 80 to 120.
CO_2 capacity 50 to 75, volume per cent.
Chlorides: total 570 to 620.
Calcium serum 9 to 11.
Bilirubin 0.1 to 0.25 mgm.
Icterus index 4 to 6.

Cerebrospinal Fluid:

Water pressure reclining 100 to 200 mm.
Children 50 to 100 mm.
(Mercury = water values × 14.) = 7-14 mm
Total amount of spinal fluid 100 to 150 cc.
Globulin a trace, not demonstrable by ordinary tests.
Cells 0 to 5.
Sugar 0.04 per cent to 0.07 per cent.
Chlorides 0.72 per cent to 0.75 per cent.

Gastric Contents. (*Adults*):

Emptying, usually in four, always in six hours.
Capacity 1 to 1½ liters. (33 to 60 ounces.)
Quantity in fasting stomach: 0 to 50 cc. 100 cc. (3½ oz.) is the extreme top limit.
One hour after test breakfast 50 to 100 cc.
Free hydrochloric acidity: fasting 5 to 30 (pH 2.4 to 1.5).
One hour after meal 20 to 60 (pH 1.7 to 1.3).
Total acidity fasting 15 to 45.
One hour after meal 30 to 75.

Urine:

Quantity: adult 1000 to 1500 cc. (33 to 60 oz.) in twenty-four hours.
Infants 200 to 400 cc. in twenty-four hours.
Children three to six years: 400 to 600 cc. in twenty-four hours.
Night amount one-quarter to one-half the day quantity.

Specific gravity: of mixed twenty-four-hour specimen 1.015 to 1.020.
After drinking much water 1.002.
After twelve hours water starvation 1.030 to 1.035.

Urine—(Continued)

Reaction: usually acid, sometimes, neutral or alkaline. Varies with diet.

Albumin: 25 to 75 mgm. per liter, too little to show with ordinary tests.

Sugar: 0.05 to 0.2 per cent, too little to show with ordinary tests.

Chlorides: 10 to 15 gm. in twenty-four hours; varies with diet.

Urea: 2 to 4 per cent.
In twenty-four hours 25 to 40 gm. Average 33.
In women 20 to 35 gm.

On diet rich in albumin, 100 gm.
In hunger or nitrogen poor diet 15 to 20 gm.
With specific gravity about 1.004 = 1 per cent urea (approximately).
With specific gravity 1.014 − 1.020 = 1.5 per cent urea (approximately).
With specific gravity 1.020 − 1.024 = 2 per cent to 2.5 per cent urea (approximately).
With specific gravity 1.028 = 3.5 per cent urea (approximately).
Urea × 1.136 = nitrogen quantity.

Phosphates 2 to 3 gm. in twenty-four hours.

Phenol red excretion one-half hour after intravenous injection, over 45 per cent.

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LABORATORY MEDICINE

A GUIDE FOR STUDENTS AND PRACTITIONERS

BY
DANIEL NICHOLSON, M.D.
MEMBER OF THE ROYAL COLLEGE OF PHYSICIANS, LONDON; ASSISTANT PROFESSOR OF
PATHOLOGY, UNIVERSITY OF MANITOBA; ASSISTANT IN PATHOLOGY,
WINNIPEG GENERAL HOSPITAL

ILLUSTRATED WITH 108 ENGRAVINGS AND A COLORED PLATE

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TO

THOSE WHOSE ORIGINAL INVESTIGATIONS

HAVE MADE POSSIBLE THE USEFUL DIAGNOSTIC PROCEDURES

HEREIN OUTLINED

26056

PREFACE.

The aim of this book is to provide, in detail, information on the indications, method and interpretation of useful tests that a medical practitioner should be able to perform. It also outlines the principles and interpretation of the more highly technical diagnostic procedures which may be performed for him at a large laboratory.

The viewpoint is the result of being engaged for ten years in teaching students clinical laboratory work. Many useful procedures have been learned at other hospital laboratories on this continent and in Great Britain and Europe. After personally trying out various methods for their comparative values, a selection has been made of the tests which are most useful in diagnosis and which can be quickly done with a minimum of apparatus. Many of them are really bedside procedures.

Finally the very rigid test of trying them out in actual practice has been made. During medical college vacations the writer has taken over busy general practices, both rural and urban. This gave an opportunity to determine the character of laboratory work that could be done under the exigencies of practice. From this experience attention has been concentrated on teaching procedures that would be of value to the student when he later engages in practice.

I am indebted to Dr. Charles Hunter for helpful suggestions on hemorrhagic diseases; to Dr. J. D. Adamson for help on the interpretation of anemias; to Dr. C. R. Gilmour for his clinical classification of nephritis; and to Dr. William Boyd, head of this department, for valuable suggestions and the opportunity to develop this book.

D. N.

WINNIPEG, CANADA.

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INDICATIONS FOR LABORATORY TESTS.

The great number of laboratory tests ordered in the large hospitals is a striking contrast to the very few done outside these centers. Probably both are too extreme. In the large hospitals there are, without doubt, a greater number of obscure cases, but the fact that a clinician can have any test done merely by ordering it tends to make too many tests routine, and the results are so constantly negative as not to warrant the time and expense involved. The clinician should have an idea of what he expects from a test. Laboratory tests are merely an extension of our special senses. The microscope detects what the naked eye cannot, but the reverse is also true. The more accomplished the clinician, the more discriminating is he in the use of the laboratory.

The only justification for doing tests not clearly indicated and the interpretative value of which is not settled by past experience is for research purposes. For research any investigation must be done with the understanding that results have no immediate diagnostic value. Accurate records must be kept and the results and conclusions published. Failing this, the end does not justify the work done.

In private practice some clinicians are so fully occupied in examining their patients that they do not have time to do the necessary laboratory tests on them. For such, the laboratory tests may be done by a nearby clinical pathologist or the clinician may employ a trained laboratory technician who will perform the reactions under his direction. If he does this it is essential that he be thoroughly acquainted with all the procedures and supply normal controls, and check tests to make certain in his own mind that no error occurs.

In medical diagnosis it is important to maintain a proper balance between the patient's mental state, the history, the physical examination, x-ray investigation and laboratory tests.

LABORATORY EXAMINATIONS INDICATED IN ALL CASES.

(In Order of Importance.)

Urine examination; general and microscopic.
Hemoglobin.

Opinion is divided as to whether the following tests should be part of a routine examination. The available facilities and the question of cost usually enter into the decision.

Wassermann test in chronic disease.
Red cell and white cell count.
Blood group determination in surgical cases.
Stool for blood and eggs of parasites. (This is a necessity in warm climates.)
Nose and throat cultures on children; any sputum present for organisms.
Vaginal smears on children entering an institution.

CHOICE OF TESTS IN INFLAMMATORY DISEASE.

INDICATIONS.

Pain.

Temperature or rarely, appearance of shock with sub-normal temperature.

TESTS.

Microscopic examination of urine for pus and bacteria (page 274.)
Differential percentage of leucocytes. (Page 56) or
Cell sedimentation time. (Page 77.)

To find out organisms:

Blood culture. (Page 192.)
Agglutination tests. (Page 191.)

If an abscess forms, puncture or incise it to obtain pus for examination by smear and culture.

PROCEDURE FOR EXAMINATION IN ANEMIA.

Indications for Examination.—

Weakness.
Dyspnea.
Dizziness.
Tinnitus.
Pallor.
Edema.
Palpitation.
Heart murmurs.

Choice of Tests to Determine if the Blood is Normal.—A hemoglobin estimation comes first. Very rarely is the blood pathological with the hemoglobin normal.

Examination of the stained or even the unstained smear will detect these exceptions. If both are normal one need not examine further for anemia.

If the hemoglobin or red cells in the smear are abnormal do: (1) red cell count or cell volume estimation; (2) a total and differential white cell count; (3) an icterus index estimation.

The cell volume percentage has much the same significance as a red cell count; the cell sedimentation time, as a total leucocyte count; and the clot contraction time as the platelet count.

The following are of occasional value:

1. Average cell diameter by the halo test.
2. A count of the reticulocyte percentage.
3. Peroxidase stain.
4. Red cell fragility.
5. Platelet count.
6. Count of cells in the saliva.

If anemia is present, search out one or more causes listed on page 95.

If the anemia is severe, determine the patient's blood group and match his blood directly with that of an available donor.

CHOICE OF TESTS TO DETERMINE THE CAUSE OF ABNORMAL HEMORRHAGE.

Multiple hemorrhages are usually due to a hemorrhagic dyscrasia. A single focus of hemorrhage is likely due to a local cause and surgical measures should be employed.

Of most value in the diagnosis of hemorrhagic disease is:

A blood-smear examination. (Page 35.)

Look for the presence of platelets, the excessive or abnormal white cells of leukemia and the irregular and scanty red cells of severe anemia.

More exact information may be obtained by:

A count of the blood platelets (page 73) of the white blood cells (page 22) and of the red blood cells (page 30).

Other tests of value are:

The time of clot contraction. (Page 180.)
The Hess capillary test. (Page 197.)
The bleeding time. (Page 177.)
The coagulation time. (Page 178.)
The icterus index. (Page 127.)

Always determine the blood group immediately and match the patient's blood directly with the blood of an available donor in case a transfusion be urgently required.

INDICATIONS FOR BLOOD CHEMICAL TESTS.

(Largely from Chemical Tests on the Blood by Rockwood, Jour. Am. Med. Assn., 1928, 91, 157.)

A full blood chemical examination is a needless expense unless there are specific indications for each test.

Order blood sugar determinations in cases only of, diabetes or suggestive subjective symptoms, with obesity, arteriosclerosis or gall bladder disease. Suspected hypoglycemia is also an indication for the test.

A sugar tolerance test is unnecessary when the fasting blood sugar is above 0.15 per cent.

Order an estimation of the CO_2 combining power of plasma (or when this is not feasible use some of the indirect tests listed on page 159) in:

1. Diabetic patients with much diacetic acid in the urine.
2. Uremic patients with much nitrogen retention.
3. Patients who are receiving large doses of alkali and showing toxic symptoms.
4. Tetany of all types.
5. Patients showing severe toxic symptoms associated with marked disturbance of gastro-intestinal motility such as occurs in protracted vomiting, peritonitis, distention or probable intestinal obstruction. Blood urea and blood chloride estimations are also of value in acute disturbance of the gastro-intestinal motility with toxemia.

Except in an emergency, never ask for a blood urea estimation when the phenolsulphonephthalein is normal. Determine the phenolsulphonephthalein first.

Never ask for both a blood urea and non-protein nitrogen; they have the same significance.

Do not ask for a blood creatinine unless the urea nitrogen is over 30. It should then be done as a routine.

Order uric acid only in cases of gout or suspected gout. Renal impairment detracts from its diagnostic value.

Order icterus index only in cases of jaundice and anemia.

Ask for blood calcium determinations only in tetany of unknown origin.

Order inorganic phosphorus tests, if practicable, in cases only of rickets or infantile tetany.

Ask for a blood cholesterol estimation only to confirm a diagnosis of nephrosis or to determine the severity of diabetes. Rule out other states in which it is increased.

PROCEDURE TO DETERMINE GASTRIC FUNCTION.

INDICATIONS.

Epigastric distress or pain or tumor.
Gas eructation. Vomiting.
Diarrhea. Loss of weight.

TESTS.—Determine the emptying time of the stomach by aspirating the contents of the stomach four or more hours after a meal. (Page 263.)

Note the appearance and odor.
Estimate the free hydrochloric Acid content. (Page 264.)

AND IF ABSENT

Repeat the examination one to two hours after a test-meal.
Examine the stool for occult blood. (Page 323.)

CHOICE OF TESTS TO DETERMINE LIVER FUNCTIONS.

INDICATIONS FOR LIVER FUNCTION TESTS.

Intolerance to fatty foods; distress immediately after eating.
Pain or tenderness in right upper quadrant of the abdomen.
Jaundice in sclera of eyes and skin.
Ascites.

TESTS FOR COMMON USE.

Examine the urine for presence of bile pigments. (Page 315.)
Take the icterus index of the blood. (Page 127.)

TESTS OCCASIONALLY OF VALUE.

Bleeding time. (Page 177.)
Examination of the feces for bile pigments. (Page 325.)
Examination of duodenal contents for pus, bacteria and crystals. (Page 272.)
Urobilin in the urine. (Page 316.)
Bromsulphthalein test. (Page 131.)
Van den Bergh reaction. (Page 131.)

Before operation always determine the blood group and match the patient's blood directly with the blood of an available donor.

TESTS TO DETERMINE INTESTINAL DISEASE.

INDICATIONS.

Diarrhea.
Malnutrition.
Constipation.
Abdominal distress or pain.

CHOICE OF TESTS.

Carmine capsule. (Page 321.)
Occult blood in feces. (Page 323.)
Microscopic examination of feces. (Page 331.)

Other procedures of value are x-ray examination following an opaque meal or enema.

Culture from lesions seen through proctoscope.

CHOICE OF TESTS TO DETERMINE RENAL FUNCTION.

INDICATIONS FOR FUNCTIONAL RENAL TESTS.

Hypertension.
Edema.
Nocturia or morning urine with a specific gravity under 1.015.
Albumin or casts in urine.
Before prostatectomy.

TESTS.—To detect early disease use the urine concentration and water dilution tests. (Page 303.)

In chronic disease which is moderately or markedly advanced use the phenol red test. (Page 306.)

In advanced disease, to determine prognosis or surgical risk test for blood urea retention by,

Mercury combining power of blood. (Page 146) or
Mercury combining power of saliva. (Page 358.)

CHOICE OF TESTS IN URETHRAL INFECTIONS.

IN MALE.

ACUTE DISEASE.—Microscopic examination of urethral discharge for cells and bacteria. (Page 202.)

CHRONIC DISEASE.—Microscopic examination of urethral discharge, two-bottle test, prostatic secretions. (Page 207.)

IN FEMALE.

ACUTE OR CHRONIC DISEASE.

Preliminary examination—urine, for pus. (Page 274.)
Two-bottle test. (Page 207.)

If pus be present, especially in the first specimen or if infection be suspected, examine microscopically a film of discharge from the urethra, Skene's ducts and the cervix uteri.

CHOICE OF TESTS FOR PUS DISCHARGES.

Smear of exudate. (1) For pus cells; (2) for organisms. (Page 202.)
Culture for special characteristics. (Page 217.)

CHOICE OF TESTS ON PUNCTURE FLUIDS.

PURULENT.
  Smear for cells and bacteria. (Page 202.)
  Culture for bacteria. (Page 215.)
CLEAR.
  Specific gravity. (Page 287.)
  Rivalta's test.
  Albumin content.

WHEN TO DO CUTANEOUS PROTEIN TESTS.

In hay fever with seasonal variation.

In young individuals suffering from spasmodic asthma.

In infants with eczematous urticaria. (Page 352.)

Cutaneous reactions to proteins WILL RARELY OCCUR IN PATIENTS OVER FORTY YEARS OF AGE WHO HAVE PROLONGED ATTACKS OF ASTHMA WHICH ARE WORSE: (1) ON EXERTION; (2) DURING ATTACKS OF BRONCHITIS ESPECIALLY IN WINTER.

It is very rare to obtain help from cutaneous protein tests in urticaria or eczema in adults.

IN HYPERTENSION.
  Blood-pressure estimation.
  Urine examination, general and microscopic.
  Water concentration test.
  If specific gravity is below 1.025, estimate the blood urea.
  Repeat these estimations after one week's medical treatment.

DIABETES.—Examine the urine at once for sugar and diacetic acid. (If diacetic acid is present, give 1000 cc. of fluids, broth, coffee, tea and water every six hours and 2 level teaspoons of granulated sugar every two hours during the day. (1 teaspoon = 4 gm. approximately.) Insulin is of life-saving value.)

Take blood sugar immediately and repeat the following morning for the fasting value. Later it may be estimated once or twice weekly if necessary.

URINE.—Daily twenty-four-hour specimens. Examine for sugar and if present estimate the total amount of sugar in grams and examine for the presence of acetone bodies.

CHOICE OF TESTS IN DISEASE OF THE NERVOUS SYSTEM.

Spinal fluid examination.

If clear examine for cell increase, globulin increase, Wassermann. (Page 236.)

If turbid examine smear for cells and bacteria. (Page 202.)

Culture for bacteria. (Page 25.)

COMATOSE STATE OR CONVULSIONS.

Intracranial disease is the commonest cause of coma and convulsions in adults while in children the acute general infections are an equally frequent cause.

VALUABLE TESTS.—Lumbar puncture and examination of the CEREBROSPINAL FLUID for pressure. If the fluid be clear examine for cells, globulin and Wassermann. (Page 236.)

If the fluid be cloudy examine a film for the type of bacteria and if necessary culture. (Page 202.)

THE BLOOD.—Stippling of red cells for lead poisoning. (Page 50.)

Blood urea for uremia. (Page 146.) (It may be twice normal in terminal infections.)

Blood sugar for diabetes. (Page 135.)

THE URINE.—In the comatose the urine examination is frequently misleading. Albumin and even red blood cells frequently occur in comas or convulsions from causes other than uremia. Sugar may be present in the urine after a head injury. Ketone bodies may occur from simple starvation. THERE IS USUALLY EDEMA AND A HISTORY OF KIDNEY DISEASE IN UREMIA AND A HISTORY OF PREVIOUS DIABETES IN DIABETIC ACIDOSIS.

Passing a catheter in elderly men may reveal a prostatic obstruction which arrests kidney secretion.

Examination of the fundus of the eye by the ophthalmoscope for papilledema, optic atrophy, arteriosclerosis or hemorrhage, albuminuria or diabetic retinitis is a most valuable procedure.

FREQUENT CAUSES OF COMA OR CONVULSIONS ARE,

A HEAD INJURY OR A BRAIN DISEASE such as meningitis, encephalitis, abscess, tumor, hemorrhage, syphilitic disease, epilepsy.

A GENERAL INFECTION by one of the communicable diseases. This group is more important in children and diarrhea and the tetany of rickets are also frequent causes.

A POISON such as alcohol, lead, morphine, carbon monoxide, uremia, eclampsia, diabetic acidosis.

CARDIOVASCULAR DISEASE, simple fainting, (under forty years of age), HEART DISEASE, cerebral arteriosclerosis which may be associated with thrombosis, embolism, or hemorrhage (over fifty years of age).

LABORATORY MEDICINE.

CHAPTER I.

THE BLOOD.

LEUCOCYTE COUNT.

Principle of the Test.—Using a pipet, the blood is diluted by an acid fluid which renders the red cells invisible and makes the nuclei of the leucocytes stand out. After thoroughly shaking the pipet to obtain an even distribution of the leucocytes, the solution is poured into a chamber of accurate dimensions. When the cells settle on the bottom, which is ruled, they are counted under the microscope. Their total is multiplied by a factor to make up for the dilution and for the fraction of 1 cu.mm. counted in the chamber. Thus a number of leucocytes in 1 cu.mm. of whole blood is computed.

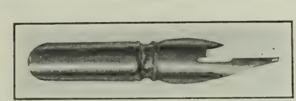

Fig. 1.—A pen nib with one blade broken off and the other sharpened chisel-like makes a good lancet to obtain capillary blood.

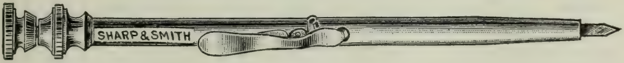

Fig. 2.—Automatic blood lancet.

Apparatus.—Microscope.—Hemocytometer with new Neubauer ruling. A single chamber is satisfactory.[1]

Leucocyte pipet with "11" mark above bulb.[2]

Diluting fluid, 2 per cent acetic acid.[3]

Spring blood lancet.[4]

Acetone or, absolute alcohol followed by ether, absorbent or gauze.

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[1] The Levy-Hausser type with Bakelite holder is very durable. The U. S. Bureau of Standards stamp at an extra cost of $4 insures accuracy.

[2] These may be had for a slight extra cost with Bureau of Standards stamp, which guarantees accuracy. The rubber tubes which usually come with the pipets are too short. Two should be joined together or a rubber tube about 1 foot long substituted. A hypodermic needle, 25-gauge, used as a mouthpiece makes an excellent throttle to fill the pipet accurately.

[3]

Acetic acid, glacial	2.0 cc.		38.0 min.
Mercuric chloride	0.1 gm.	or	1.5 gr.
Water to	100.0 cc.		4.0 oz.

The mercuric chloride will prevent growth of moulds and the solution lasts indefinitely. A 1-oz. bottle is filled for use. It should be filtered frequently, as some cells are deposited in it each time a pipet is immersed.

[4] A pen nib with one point broken and the other sharpened chisel-like, or a hypodermic needle about gauge-22 makes a cheaper but much less satisfactory substitute.

OUTLINE OF TECHNIQUE FOR THE LEUCOCYTE COUNT.

1. OBTAINING THE SPECIMEN.—Obtain a large drop of blood before collecting any. Draw blood to 0.5 in the pipet and dilute to "11" with 2 per cent acetic acid. Shake well. Discard the first drop. Place the diluted blood in the counting chamber. It must not flow into the trench.

2. THE COUNT.—Use the low-power lens and closed diaphragm. Count all cells in the four corner squares, each made up of 16 small squares and multiply the total by 50. Normal, 4000 to 12,000 per cu.mm.

3. CLEANING PIPETS.—Clean pipets immediately after use. Blow out any cells remaining in the bulb. Fill twice and empty (1) with water, (2) with acetone. Draw air through to dry.

TECHNIQUE IN DETAIL.

1. **Obtaining the Specimen.**—Have the 2 per cent acetic acid bottle uncorked and a clean, dry leucocyte dilution pipet connected with the rubber tube.

Clean the lobe of the ear and the needle point with alcohol and allow to dry. Set the lancet to penetrate 2 mm. (between $\frac{1}{16}$ and $\frac{1}{8}$ inch) and puncture the lower edge of the ear lobe. The blood should flow, but if it does not, even after a deep stab, moderate pressure some distance from the puncture does not alter the result. In practising blood counts on one's self it is necessary to obtain blood from the finger. The ball of the finger is extremely sensitive, and it is best to puncture the back of it between the nail and the first joint, then grasp the finger in the other hand so as to encircle it like a tourniquet. When the cut finger is firmly flexed a large drop of blood at once wells out. Wipe away the first drop. **It is very essential that the drop for the test be a large one.**

Hold the pipet horizontally, at right angles to the line of vision, and suck the blood slowly up to "0.5" mark. If the column of blood has passed the mark slightly, to less than the "0.6" mark, it may be taken down exactly to the "0.5" mark by touching the tip of the pipet with the finger or cotton. The increase in count resulting from this is negligible. If too much time is taken with this the blood will clot and invalidate the result. Wipe off the blood adhering to the tip of the pipet, draw in a bubble of air and plunge it into the 2 per cent acetic acid, holding the pipet nearly horizontally and rotating it between the thumb and fingers to prevent a bubble adhering to the glass bead. Draw in quickly at first to wash all the cells from the sides of the pipet, then as the fluid fills more than three-quarters of the pipet bulb, apply the suction as gently and as steadily as possible; because where the bulb narrows to the "11" mark the fluid rushes up quickly, and may pass the mark if care is not exercised. Stop the fluid column exactly at mark "11" by merely ceasing to draw in air. Placing the tongue in the end of the mouthpiece or pinching the tube will displace the fluid out of the bulb and alter the result. Still holding the pipet in a horizontal position, place a finger over the tip, remove the rubber suction tube, place the thumb over the other end of the pipet and shake vigorously for a minute, using a side-to-side and rotary movement. If there is any error in the above procedure clean the pipet and repeat.

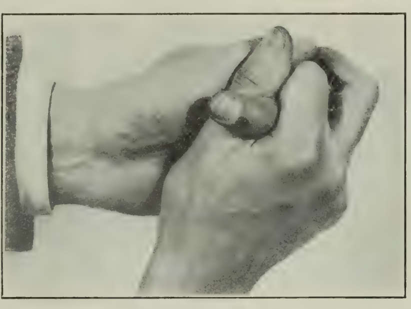

Fig. 3.—Convenient method for obtaining a drop of blood for a cell count.

The diluting fluid in the stem of the pipet up to "1" does not mix with the cells. The bulb, therefore, holds 10 units, which is the difference between "1" and "11," and 0.5 units of blood in this makes a dilution of 1 in 20. When not convenient to count the corpuscles at once, place a heavy rubber band around the pipet, so as to close the ends, and it may be carried about in a horizontal position. If the pipet is held perpendicular the cells will fall into the stem, after which they cannot be properly mixed with the diluting fluid. The cells will keep for twenty-four hours.

2. **The Count.**—Wipe the counting chamber and cover-glass with a clean linen or cotton cloth. Place the cover-slip on the counting chamber, and, *after thoroughly shaking the pipet,* blow out a drop which is the unmixed diluting fluid in the tube. Discard this drop. Wipe the tip. Hold the pipet with its tip resting on the ruled

platform and touching the cover-glass. With the left hand pinch the tube near the pipet, so as to expel a small drop which will flow under the cover-glass to cover the platform. Slightly less than this does not impair the result. If there are bubbles it indicates that the glassware is greasy and requires cleaning first with soap and water, then alcohol. If the fluid overflows into the groove many cells will gravitate into the lower area and invalidate the result.

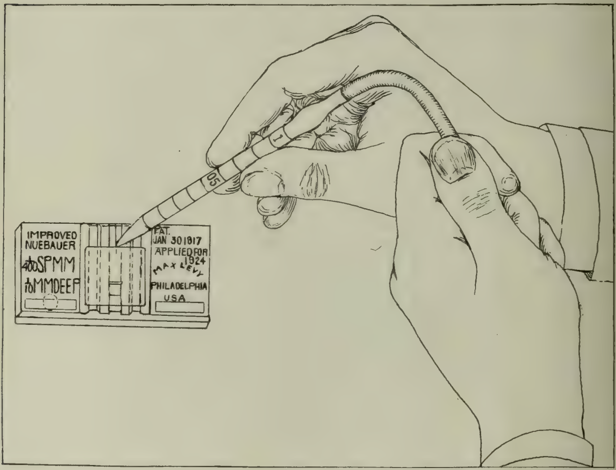

FIG. 4.—Filling the counting chamber. By rolling the thumb and finger of the left hand up on the tube, a small drop may be expelled to fill the counting chamber without overflowing.

When either of these occurs clean the counting chamber and cover-glass, and after shaking the pipet refill the chamber.[1]

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[1] *Old Types of Counting Chambers.*—Some of the old counting chambers have lines that are barely perceptible. If the lines are not clear after the light is subdued, wipe off the diluted blood, rub a soft lead pencil on paper, then apply the paper to the counting chamber, moving it with a circular motion so as to deposit the graphite into all the ruled lines. Wipe off the surface with a dry cloth and refill with diluted blood as before. Some have cemented parts and no alcohol can be used on them. Soap and water will remove grease.

The Thoma-Zeiss Type of Counting Chamber.—The Thoma-Zeiss type of counting chamber has a round disk on which the ruled lines are cut. This surface is exactly $\frac{1}{10}$ mm. below the general surface and is surrounded by a deep channel. The Thoma chamber is quite as accurate as the new types with the open end, but there is always difficulty in placing on the cell a drop that nearly covers the ruled surface, but does not flow into the channel around it. If the drop is not the right size the chamber must be cleaned and another drop put on it.

Allow one minute for the cells to settle. Before putting the eye to the eyepiece place the ruled portion of the filled counting chamber exactly at the center stage. Lower the low-power objective lens to about $\frac{1}{4}$ inch from the counting chamber. Then place the eye to the eyepiece and arrange the microscope mirror so that the brightest light possible comes through. Avoid direct sun rays. Now close the diaphragm to admit a minimum of light and focus up until the ruled lines and cells are clearly seen. By focussing *only upward* one avoids all possibility of lowering the objective to break the cover-glass.

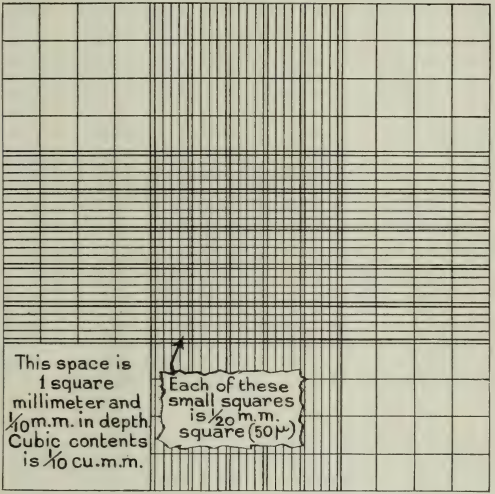
This space is 1 square millimeter and 1/10 m.m. in depth. Cubic contents is 1/10 cu.m.m.
Each of these small squares is 1/20 m.m. square (50 µ)

FIG. 5.—Improved Neubauer ruling on a hemocytometer.

The cells appear as small granular balls.

Place the corner square under the low power of the microscope and count in uninterrupted succession the cells in the sixteen squares. Corpuscles which touch the upper and left sides should be counted. This avoids counting cells in a greater area than the square or counting the same cells twice. Count the corresponding square at each corner. The counts on each corner should not vary more than 12 cells. If they do it is because the cells were not thoroughly mixed in the bulb of the pipet, Multiply the total cells in the four squares by 50.[1] Shake up the remaining solution in the pipet, put it in the counting chamber and repeat the count. Take the average of both counts.

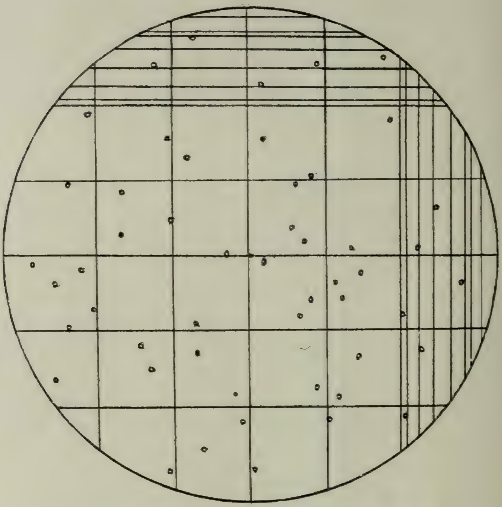

Fig. 6.—Leucocytes in the hemocytometer under the low-power lens of the microscope. Twenty-six leucocytes appear in the area made up of 16 small squares. An average of this for 4 or more similar squares would give a leucocyte count of 5200 per cu.mm.

3. **Cleaning the Counting Chamber and Pipet.**—Immediately after completing the count clean the counting chamber and the diluting pipet. Wash off the counting chamber and cover-glass with water, then wipe with alcohol or acetone to remove any grease and dry with a clean cloth. Blow out any solution remaining in the bulb of the pipet. Then wash by filling twice in the following order; with water, acetone and air or with water, absolute alcohol, ether and air. A rubber hand bulb or suction pump when available does this with least effort but the rubber tube and mouth suction is satisfactory provided one shakes out the ether or after blowing out

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[1] Each of the 4 squares is 1 mm. square and 0.1 mm. in depth. This makes a cubic content of 0.1 cu.mm. for 1 square and 0.4 cu.mm. for the 4. The blood is diluted 20 times. Therefore multiply by 20 for the blood dilution and by the 0.4 cu.mm. is of 1 cu.mm., namely 2.5, 20 × 2.5 which gives the multiple of 50.

the ether *draws* in an air current until the bead rolls freely in the bulb without tending to adhere to the sides. If *blown* through, the moist expired air will form, with any ether that remains, a sticky opaque film inside the bulb. Fill the pipets occasionally with strong acid and leave standing overnight to remove any traces of blood protein that may remain adhered to the walls. Then wash thoroughly with water to remove the acid. Any attempt to remove a clot from the pipet tip by means of a needle or wire usually chips the tip and renders the pipet inaccurate.

Soften the clot in alkali. Then use a horsehair such as is used for ligature or coat interlining to push out obstructions in the stem of the pipet. After this fill the pipet with acid to clean thoroughly.

The Normal is 4000 to 12,000 leucocytes per cubic millimeter.

The pipet dilutions give most difficulty and should be practised with colored water before trying blood. Doing daily blood counts on oneself is an excellent method of acquiring skill. Many can acquire a good technique in two weeks while others require longer. Consecutive counts should not vary more than 2000 in the case of leucocytes and 200,000 in the case of red cells. They should also be checked with the counts done by a skilled technician. If you have not done a blood count for several months it is best to count your own leucocytes for practice just prior to doing an important leucocyte count on a patient. This also insures that all equipment is in working order.

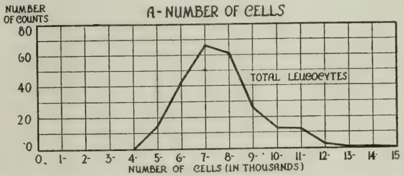

FIG. 7.—Total leucocyte counts, varying from 4 to 12 thousand with the highest number at 7. Analysis of 241 counts from 17 individuals. New York City. (Medlar: Am. Jour. Med. Sci., 1929, **177**, 72.)

4. **Interpretation.**—*The Count in Health.—In health* the count varies from 4000 to 12,000 in persons who are normally active. Four thousand to 9000 is the variation at rest in bed on a light diet. Its highest point is in the afternoon, just as the body temperature is a degree higher in the afternoon than the forenoon. The maximum count of an individual is usually twice their minimum.

The Count in Disease.—In acute infections repeated counts at the same hour daily or every four hours in serious cases should be done to note the trend of the fight between the infection and the resistance.

1. *A count of* 3000 *to* 12,000 *with a high fever* occurs in an acute bacillary infection such as typhoid or influenza. The count is usually nearer the lower limit.

2. *A count of* 3000 *to* 12,000 *in a very ill patient* who has normal or subnormal temperature, rapid pulse, cold extremities and a localized pain is usually the result of a virulent infection which has temporarily overcome the bodily resistance. An example of this would be a fulminating attack of appendicitis. The differential count is usually of value here. The extent to which the polymorphonuclears are over 75 per cent show the severity of the infection. Over 80 per cent of polymorphonuclears may be found in severe infections and over 90 per cent in the worst type of infection. The increase in the young polymorphonuclears with the unsegmented U-shaped nucleus also indicate the extent of the call on the bodily resistance. The normal percentage of such cells is 5. In moderate infection it may go up to 10, in severe infections, up to 20, after which time a few myelocytes appear. This indicates a state of dangerous illness. With infections of long-standing such as arthritis the count is usually normal. The absence of eosinophiles is of serious import.

3. *A count of* 12,000 *to* 16,000 *with fever* is usually found in mild or moderate infections, often streptococcal, and where there is a satisfactory resistance. Trivial infections, such as a mild tonsillitis, will cause a leucocyte count of 12,000. With a leucocyte count of over 15,000 there is almost always pus formation. In a malignancy the leucocytosis is due to infection. Although usually the leucocytosis is moderate, it may rarely be very high.

4. *A count of* 16,000 *to* 25,000 *with high fever* accompanies virulent infections due to streptococci, pneumococci or other cocci in subjects who have a good resistance. There is always pus formation with such a leucocytosis and the symptoms and signs indicate the site of the lesion. The degree of leucocytosis indicates the vigor of the resistance, but as this is whipped up to the degree required to cope with the infection the degree of leucocytosis also comes to indicate the severity of the infection. Usually the leucocytes hold the infection at bay for several days until immune bodies are developed and brought by the plasma to check the development of the infection after which the mononuclear phagocytes digest the inactivated bacteria and débris. Continued leucocytosis means extension of the infection. If the leucocyte count is over 30,000 a part

of the resistance mechanism may fail, causing a fatal outcome. Very high leucocyte counts in pneumonia do not carry a bad prognosis. Low counts do.

One should not overlook the possibility of hemorrhage when seeking the cause of a leucocytosis. It has some very special features.[1] Leucocytosis only occurs with hemorrhage into a serous cavity as the peritoneum, pleural cavity or intradurally. The rise of the leucocyte count starts an hour or two after the hemorrhage. It reaches its maxium, which is one and a half to three times the normal, in six to ten hours and returns to normal again four days after the hemorrhage has ceased.

5. A count of 25,000 or over with little or no fever suggests leukemia or glandular fever, rather than a pyogenic infection. The higher the count the more certain the diagnosis of leukemia. The highest counts are rarely above 30,000 in glandular fever. In leukemia the white cell counts are usually much higher than 30,000. Glandular fever or infectious mononucleosis has no accompanying anemia and is a benign disease. In the leukemias, which are always fatal, there is always a profound anemia and examination of the smear will tell whether it is lymphatic or myelogenous.

THE RED CELL COUNT.

PRINCIPLE OF THE TEST.—The blood is diluted by a fluid which preserves all the cells. The cells of the diluted blood are counted in a chamber as in the white cell count. Both red and white cells are counted but as there are about 1000 red cells to one white, the white cells do not materially change the result. The total number of red cells per cubic millimeter of undiluted blood is estimated by multiplying by a factor to make up for the fraction of a cubic millimeter counted and for the dilution.

Apparatus:

Microscope.
Red cell pipet marked "101."
Hayem's solution as a diluting fluid.[2]
A spring lancet.
Acetone (or alcohol and ether), absorbent or gauze sponges.

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[1] Wright and Livingston: The Leucocytosis of Internal Hemorrhage, New York State Med. Jour., 1923, **23**, 286.

[2] *Hayem's Solution.*

Mercury bichloride	0.5 gm.	or	5 gr.
Sodium chloride	1.0 gm.		9 gr.
Sodium sulphate	5.0 gm.		46 gr.
Distilled water	200.0 cc.		4 oz.

This solution does not deteriorate. Filter and place some in a 1-oz. bottle for use. Cells which fall from the tip of the pipet should be removed by occasional filtering. If this solution is not at hand fair results may be obtained by using as a diluting fluid 1 dram of table salt to 1 pint of fresh water.

OUTLINE OF TECHNIQUE FOR THE RED CELL COUNT.

1. OBTAINING THE SPECIMEN.—Draw blood to 0.5. Dilute with Hayem's fluid to 101. Shake well. Discard the first drop. Place the diluted blood in counting chamber. It must not overflow.

2. THE COUNT.—Use the high dry lens. Count 5 large squares each including 16 small squares. To the total of cells add four zeros. This is the number of cells per cubic millimeter. Normal 4 to 6 million.

3. CLEANING PIPETS.—Clean pipets immediately after use. Blow out any cells remaining in the bulb. Fill twice and empty, (1) with water, (2) with acetone (3) draw air through to dry.

TECHNIQUE IN DETAIL FOR THE RED CELL COUNT.

1. **Obtaining the Specimen.**—Except that the red cell pipet marked "101" and Hayem's diluting fluid are used, the same technique is used as for the leucocyte count. The blood should be fresh and there should be a normal blood circulation to the area with no edema, cyanosis or even coldness. Tourniquet-like pressure above the wound does not vitiate the results but undue pressing about the incision lowers the count. Draw the blood up exactly to 0.5 and after wiping the tip of the pipet, draw in the Hayem's diluting fluid to the "101" mark. It is very essential to have the correct amount of blood. Shake the pipet immediately after it is filled to prevent any clumping of the cells. The cells will keep in the solution for twenty-four hours. The pipet should be maintained in a horizontal position or the cells will gravitate into the stem.

2. **The Count.**—After shaking the pipet both with a rotary and side-to-side motion, blow out the contents of the stem of the pipet which is only diluting fluid and place a drop from the pipet bulb at the margin of the cover-slip and counting chamber so it will run into the chamber by capillary action as described under the leucocyte count. Place the ruled portion of the hemocytometer on the center of the microscope stage and, looking at the microscope from its side, lower the low-power objective lens close to the cover-glass of the counting chamber at the point where the ruled lines are. Adjust the mirror to give the maximum of light, then close the diaphragm to give a little more than the minimum and focus up slowly until the lines come into view. The cells should be evenly distrib-

uted. If they are not, replace them by a well-mixed drop. Turn in the high dry lens and looking at the microscope from its side lower the lens objective until it almost touches the cover-glass. Open the diaphragm slightly and focus up slowly until the lines come into view.

Bring a corner square of the closely ruled center area into view under the microscope and *count in uninterrupted succession all the cells in the* 16 *smaller squares.* Include the cells touching the upper and left hand lines but do not count those touching the lower and right hand lines. A count of 100 cells in the 16 small squares would amount to 5 million per cu.mm. and 80 cells would amount to 4 million; but as this area is too small to give a representative average, count similar squares at each corner and one near the center. This makes a total of 5 large squares each made up of 16 small squares. The counts in the large squares should not vary more than 20. If they do, the cells are unevenly distributed and a fresh well-mixed drop should be counted. Add the cells counted in the 5 large

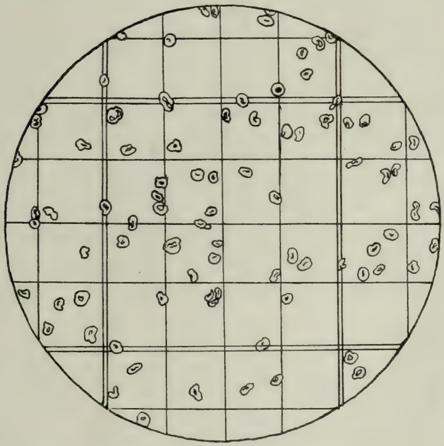

FIG. 8.—Red cells in the hemocytometer under the high dry lens of the microscope. To have a red cell count of 5 million there should be an average of 100 cells in the large square bounded by double lines and made up of 16 small squares. An average of 80 cells will give a count of 4 million. This blood is from a case of severe anemia and there are only 35 cells. Most of them are very irregular in outline and size.

squares and append 4 zeros to the total. This is the number of red cells per cubic millimeter.[1]

Caution.—The accuracy of a count depends on a number of factors. Both the pipet and counting chamber should be accurate. Only a few of those sold are inaccurate but it is best to employ only apparatus checked by the Bureau of Standards or apparatus found to give the same result as that approved by the Bureau of Standards. Most of the other sources of error are personal ones such as, an inaccurate column of blood, too small a drop of blood which allows bubbles in the column, undue delay before diluting the blood which allows clotting, insufficient shaking which gives an uneven distribution of cells, placing on the platform too much fluid which goes over into the grooves at the side or using old, unfiltered diluting fluid which may contain corpuscles.

3. **Cleaning the Pipet.**—The stem of the red cell pipet has a very narrow bore and cleaning may be more readily done by placing the rubber tube on the tip of the pipet and expelling the fluids through the end of the bulb marked "101," which has a larger bore. It should then be emptied and cleaned with water, acetone and air or with water, alcohol, ether and air as described for the white cell pipet where special directions are also given for removing clotted blood.

4. **Interpretation.**

Normal number of red cells in a cubic millimeter of blood.

At birth.

Average 7.5 million.
Total range 6 to 9 million.

After eight days.

Average 6 million.
Total range 5.2 to 6.6 million.
After eight days there is a slow gradual drop to the adult figures. This is in contrast to the low hemoglobin figures at one and two years of age.[2]

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[1] Each of the 16 small squares in a large square seen under the high-power lens is $\frac{1}{20}$ mm. square and $\frac{1}{10}$ mm. deep. Therefore, the cubic contents is $\frac{1}{20} \times \frac{1}{20} \times \frac{1}{10}$, or $\frac{1}{4000}$ cu.mm. In 5 large squares there would be 80 small squares having a cubic content of $80 \times \frac{1}{4000}$ or $\frac{1}{50}$ cu.mm. The number in 1 cu.mm. would be 50 times as much, and as the blood is diluted 200 times in the pipet the number of cells in 1 cu.mm. of undiluted blood is 50 × 200, or 10,000, which is equivalent to adding 4 zeros.

[2] Mayer: Study of Erythrocyte and Hemoglobin Curves at Various Ages, Arch. Int. Med., 1922, **30**, 478.

Adult males aged nineteen to thirty years.
Average 5.4 million.
90 per cent of men 4.7 to 6.1 million.
Total range of men 4.2 to 6.4 million.

Adult females aged nineteen to thirty years.
Average 4.8 million.
90 per cent of females 4.3 to 5.3 million.
Total range of females 4.07 to 5.55 million.

The red cell counts of an individual may vary half a million in eight hours. Counts on capillary and venous blood are usually within a quarter of a million of each other in health and in most diseases, pernicious anemia being a remarkable exception where the cell counts in capillary blood may be a million higher than venous blood, due to the many macrocytes being retarded in the capillaries. (These figures were obtained at Portland, Oregon, which has an altitude not over 500 feet, by Osgood.[1] They correspond to figures obtained by Haden in the Midwest, U. S., myself at Winnipeg, many writers in the eastern United States, and Gram and Noorgand in Denmark. They can be generally accepted as normal for well nourished people.)

Decreased Red Cell Counts in Anemia.—A more detailed discussion appears under the classification of anemias.

Counts of 3.5 to 4 million may be regarded as mild anemias.

Counts of 2.5 to 3.5 million may be regarded as moderate anemias.

Counts below 2.5 million may be regarded as severe anemias.

Most patients with secondary anemia have only a mild or moderate cell decrease when they are first examined. In fact there may be only a hemoglobin decrease with the cells over 4 million in very early cases. Pernicious anemia patients have a moderate or severe cell decrease when they first consult a physician.

The red cell count is an important diagnostic sign in anemia. It is a quantitative figure that can be recorded. It is not subject to the opinion of the examiner. It gives somewhat parallel information to a blood-smear examination for anisocytosis. The examination for anisocytosis has the disadvantage that it is subject to the opinion of the examiner who may not be well acquainted with the normal and abnormal degree of variation and it is difficult to record the varying pathological degrees of anisocytosis without doing actual cell diameter measurements and charting it as a Price-Jones curve; or examining by the halo method.

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[1] Arch. Int. Med., 1927, **39**, 643.

Increased Red Cell Count in Polycythemia.—In infancy there is a normal polycythemia of 6 to 9 million. In healthy adults the red cell count increases to 8 million at high altitudes. In acute severe diarrheas, persistent vomiting, starvation or shock, the blood count is also high due to the rapid removal of the liquid part of the blood from the bloodvessels. It may be present in marasmic states in infants.

In chronic disease, an increase in the number of red cells is not uncommon. A count of 6 to 9 million may result from a chronic embarrassment to the pulmonary circulation as in mitral disease, chronic asthma with emphysema, and congenital stenosis of the pulmonary artery. Cyanosis from any cause will be attended with an increased red cell count.

Without known causes the greatest increase occurs in the Osler-Vaquez disease or polycythemia vera. The skin and mucosa have a deep reddish color and the red cells are from 8 to 12 million per cu.mm. Occasionally the blood picture may resemble myelogenous leukemia in the late stages or even end in a profound anemia if the patient lives long enough. Phenylhydrazine in 5-gr. doses daily will reduce the abnormally high cell counts but its administration should be controlled by frequent blood counts.[1]

THE BLOOD SMEAR.

PRINCIPLE.—A small drop of blood is thinly spread on a glass slide and the cells are viewed under a very high magnification lens of the microscope. A staining fluid may be used to sharpen the various outlines especially the nuclei and granules of the white cells. This is the most important SINGLE examination of the blood cells.

Apparatus and Reagents.—Microscope and lamp.
Microscopic slides and cover-glasses. These should be very clean.
Spring lancet or as a substitute a needle with a cutting edge.
Alcohol and absorbent or gauze wipes.

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[1] Hurwitz and Levitin: Am. Jour. Med. Sci., 1929, **177**, 309.

OUTLINE OF TECHNIQUE.

MAKING THE BLOOD FILM.—Place a small drop of blood near the end of one slide. Spread it out with the edge of a cover-glass or other spreader, by making the blood follow the moving spreader.

EXAMINATION MAY BE DONE ON THE UNSTAINED FILM.—Examine the film unmounted under the high dry lens and subdued light for the following features: irregularity in shape and size of the red cells; the relative frequency of white cells and the platelets may be clearly made out.

THE STAINED FILM.—For a general examination use the high dry lens WITH THE FILM MOUNTED. For minute detail use the oil-immersion lens. (*a*) Routine stain of eosin and hematoxylin for all features listed below except 5, 6 and 7, which are infrequently required. Alcoholic eosin one to two minutes. Wash. Hematoxylin two to five minutes. Wash. (*b*) Special stains of Wright or Leishman for all features listed below. Stain with 8 drops for one minute. Add an equal amount of distilled water for three to five minutes. Wash with distilled water. Allow to dry. Mount. (*c*). EXAMINE FOR THE FOLLOWING FEATURES:

1. FREQUENT AND IMPORTANT FINDINGS.—Achromia—an unusual lack of color in the center of some or all red cells.

2. Poikilocytosis—a deviation from the circular outline, not artefact.

3. Anisocytosis—an irregularity in the size of the cells (*a*) Normocytes, red cells of normal size. (*b*) Microcytes, red cells smaller than normal. (*c*) Macrocytes, red cells larger than normal.

INFREQUENT FINDINGS.

4. Nucleated red cells (*a*) Normoblasts—a cell similar in size and cytoplasm to the red cell, but having a small blue nucleus. (*b*) Microblast—a nucleated red cell, smaller than a normoblast. (*c*) Megaloblast—a large red cell with pale cytoplasm and a nucleus.

5. Basophilic stippling—minute black dots in occasional red cells.

6. Polychromatophilia—an irregularity in staining of the red cells.

7. Malarial parasites—appear in the cell as blue ring forms, crescents or bands, small or large with red or black dots in them.

IMPORTANT OBSERVATIONS OTHER THAN RED CELLS.

8. Blood platelets—occur in clumps, note approximate change in their 1 in 25 ratio to red cells.

9. White cells—more occur at the end and margin of the smear than in the center; note the approximate change in their 1 in 1000 ratio to red cells, if increased, whether lymphocytes or polynuclears.

10. Differential white cell count—see page 56.

Interpretation in Brief.—1. Achromia is the main change in hemorrhagic or chlorotic anemia.

2. Poikilocytosis occurs in any moderate or severe anemia.

3. Anisocytosis is most marked in pernicious and less so in other hemolytic anemias. It does occur in hemorrhagic anemias but not until the achromia is very marked. The Price-Jones diameter curve is a precise method of recording this observation with the halo method a valuable substitute. See graph, page 47.

4. Nucleated red cells occur in anemias where the immature cells are called out. The very severe chronic anemias like pernicious may call out the very primitive megaloblast.

5. Basophilic stippling with a red cell count from 3 to 5 million is pathognomonic, although not present in all cases, of lead poisoning.

6. Polychromatophilia is found in any severe anemia.

7. Malarial parasites mean malarial fever.

8. Platelets.—A reduction to one-quarter their normal number may be detected from the blood smear and means aplastic anemia or thrombocytopenic purpura.

9. White cells.—An increase of 10,000 to 20,000 leucocytes which usually results from infection by streptococci pneumococci or other cocci may be detected from the blood smear. The marked lymphocytosis such as occurs in bacillary infection like whooping cough may be detected. The enormous white cell increase occurring in the leukemias is readily detected in the unstained smear by anyone who can differentiate a red cell from a white cell.

TECHNIQUE IN DETAIL.

Clean glass slides are necessary for satisfactory blood smears. A few may be cleaned by soap and water, alcohol and a clean cloth. Larger numbers are more readily cleaned by methods outlined under cleaning of glassware. Just before use passing slides rapidly through a gas flame will remove any lint or moisture. The slide should not be warmer than body heat when the smear is taken. Every microscopic glass slide has one side convex and the other concave. The The convex side will spin freely on a flat surface; the concave will not. Spread blood smears on the convex surface. The concave surface rests more steadily on the microscope stage.

(*A*) **Making the Blood Film.**—Clean the skin surface of the ear lobe or finger with absorbent and alcohol. After it is dry make a slight incision with an automatic lancet or cutting-needle. Wipe away the first drop. To the second drop, when slightly larger than the head of a pin, touch a clean glass slide an inch from one end. Holding this slide by the ends between the thumb and forefinger of the left hand, bring the end of the spreader against the surface of the slide at its center so as to form an angle of 30 degrees on the side which carries the drop of blood.[1] Holding the spreader edge firmly at this angle, draw it back until the drop is reached and after the blood creeps across the end of the spreader by capillary attraction push the spreader with a steady stroke across the slide until the drop which follows is all spread out.

Sometimes it may be necessary to move the spreader from side to side to make the blood run across the end. A greater angle than 30 degrees between spreader and glass makes a thick smear which is good for a differential count. A lesser angle makes a thinner one which is good for a red cell examination. A drop that is too large gives a smear that is too thick. Even when unstained such a smear appears red and the cells are heaped together in such a manner that they cannot be properly stained or recognized. The average satisfactory preparation is made from a drop of such size that the smear extends across one-third to one-half the slide. The first part of the spread is thicker than the last part. The first part appears reddish-yellow, and under the microscope the cells should overlap slightly. This is an excellent place to view the white cells. The cells' diameters are slightly less where the spread is thick. The last part of the smear, which is thinner when unstained, appears a pale yellow and

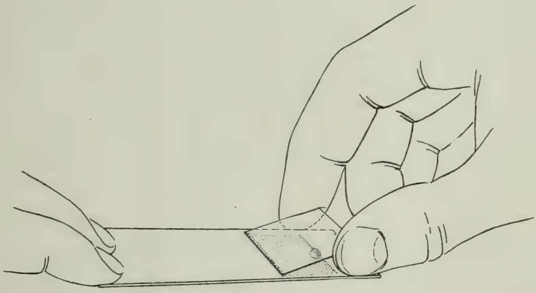

Fig. 9.—Spreading a blood film: (1) Move the spreader to the right, back into the drop; (2) when the blood spreads along the spreader, push it to the left with the blood following it.

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[1] The best spreader is a No. 2 cover-glass, 22-mm. size, which is somewhat narrower than the glass slide. The even margin and flexibility allows it to fit the slide closely. The thick cover-glass of the counting chamber is the best substitute. If neither of these is at hand the end of a glass slide will do, but the blood will extend right up to the edge of the slide where staining and examination are difficult.

under the microscope the cells are separate from each other. This is the best portion for the red cell and platelet examination. Any halt in movement of the spreader will leave a bare line in the smear. A greasy slide will cause the blood to spread irregu'arly.

Examination of the Specimen.—(*A*) *Unstained Film.*—Under the low power lens of the microscope, with the light subdued, find a place on the unstained and unmounted smear where the cells are discrete. Turn in the high dry lens and increase the diaphragm slightly. The red cells appear as greenish-yellow circles, having a center of a paler shade. Note any irregularity in outline and size. These features are discussed on page 42 and are as easily made out as on a stained smear. One cannot be certain about achromia unless it be marked.

Examination of the white cells is less satisfactory than the red cell examination in the unstained smear. In unsubdued light the white cells appear a clear refractile white in contrast to the yellowish-green tint of the red cell. The approximate frequency of white cells may be determined, especially an increase, where there are two or more white cells on every microscopic field in a thin smear. One can distinguish the lymphocytes, which are the size of the red cells, from the polymorphonuclears which are one and a half to twice their size. The nuclear outline of the polymorphonuclears may be faintly seen.

The platelets are from one-fifth to one-half the size of a red cell. They are frequently in clumps, irregularly distributed, and have a glistening appearance when the focus of the microscope is slightly moved.

The oil-immersion lens without oil or cover-slip will give a more enlarged and equally distinct but less extensive view of the cells. The use of oil or a cover-slip renders the cell outlines indistinct with the unstained dry smear.

Examination of the red cells from the unstained smear together with a hemoglobin estimation will determine whether the blood is normal or diseased. If the red cells show any abnormalities in the unstained smear or the hemoglobin is below 70 per cent, or if investigation of the white cells is required, stain the smear for further examination.

Choice of a Blood Stain.—Each stain has its advantages and its defects.

1. *Eosin and Hematoxylin* (Logwood) will last for years and almost never fail to stain. The smear may be washed with ordinary tap-water. The eosin produces an intense red in the erythrocytes

and the eosinophilic granules while the hematoxylin gives a sharp etched picture of the nuclei of the white cells and a faint staining of the mononuclear cytoplasm. It is particularly good for differential counts and is less liable to failure than any other staining process. This stain does not show granules of the polymorphonuclear neutrophiles, basophilic stippling of the red cells or malarial parasites, and platelets only very faintly.

2. *Modification of Romanowsky Stains such as Wright's or Leishman's.*—These stains show neutrophilic granules of polymorphonuclears, basophilic stippling, malarial parasites and platelets very clearly. They also give a beautiful water-color effect of the white and red cells. Their disadvantage is that they must be prepared with special, pure absolute methyl alcohol. They deteriorate slowly. Distilled water, near the neutral point, must be used to dilute the stain; the glass slides must have no traces of chemicals on them and a certain amount of special skill is required to obtain good staining results. It is better to buy these stains already prepared in 1-ounce bottles and transfer the stain to the slide with a clean dry medicine dropper.

For routine work I prefer eosin and hematoxylin and for special examinations, such as stippling or malarial parasites, a Romanowsky stain.

(*B*) STAINING THE FILM FOR ROUTINE EXAMINATION.—PRINCIPLE OF STAINING WITH EOSIN AND HEMATOXYLIN.—The eosin is made up in absolute alcohol which fixes the cells as the eosin stains the erythrocytes and eosinophilic granules. The hematoxylin stain which follows stains the nuclei of the white cells.

Reagents and Apparatus.—Alcohol eosin solution (eosin, alcohol soluble, 0.1 gm., absolute methyl alcohol, 50 cc. The methyl alcohol is a better fixative than ethyl alcohol.)

Hematoxylin.—Delafield's is best but any of the standard prepared solutions, such as Ehrlich's, work well and keep indefinitely. In preparing them they require several weeks ripening so if a prepared one is not at hand Harris hematoxylin may be prepared for use immediately as follows:

Harris Hematoxylin.—Dissolve 1 gm. hematoxylin crystals in 10 cc. of 90 to 95 per cent alcohol. Dissolve 20 gm. of ammonia alum in 200 cc. of hot distilled water and add the hematoxylin which has previously dissolved in the alcohol. Boil the mixture and after removing it from the heat add 0.5 gm. mercuric oxide. After two minutes plunge the vessel into cold water. Filter before use. It is more satisfactory for blood smears with the usual acetic acid omitted.

Keep the stains in rubber-stoppered cylindrical vials 3½ inches high and with a mouth and inside diameter 1½ inches, which will admit a microscopic slide. Fill with the stain to 2½ inches, which leaves ½ inch of the dipped-in slide to grasp. Filter frequently.

STAINING WITH EOSIN AND HEMATOXYLIN.—1. Immerse the slide in the vial containing the alcoholic solution of eosin for one to two-minutes. Wash with tap-water.

2. Immerse the slide in the vial containing hematoxylin for three to five minutes. Wash. Dry and mount with Canada balsam under a cover-glass and label.

Important Points.—If the red cells are crenated or wash off the slide the alcohol in the eosin solution is probably not absolute methyl or is diluted with water. This occurs readily with thick blood spreads. If it occurs with ordinary spreads heating the spread as hot as the hand can stand will fix the cells. If the red stain is in between the cells it speaks for poor washing after the alcoholic eosin.

If the red cells are not well stained the eosin should be stronger or the staining time longer. If the nuclei of the white cells are not a deep purplish-black the hematoxylin is at fault and if five to eight minutes of staining does not remedy the defect the stain must be replaced by a potent one. Flakes of black material over the blood film if present are the result of a precipitate in the hematoxylin which filtering will remove.

Special Stains.—It is best to purchase the prepared stains in 1-ounce bottles from a reliable chemical house.[1] If the stain is prepared in the laboratory by dissolving the powdered stain in alcohol it is necessary to use glassware chemically clean and special methyl alcohol for staining purposes that is acetone-free.

To make up Wright's stain dissolve 0.1 gm. of the powdered stain in 60 cc. of special absolute methyl alcohol.

For Leishman's stain dissolve 0.15 gm. of the powdered stain in 100 cc. of special absolute methyl alcohol.

The staining process is the same for each.

PRINCIPLE OF STAINING WITH WRIGHT'S AND LEISHMAN'S STAIN.—The alcoholic solution of stain fixes the cells on the slide and as the stain is less soluble in water than alcohol an even coat is precipitated on the cells when the water is added.

1. So that the slide may be washed without being moved, place it over a bowl or staining receptacle.

2. Stain for one minute with 8 to 10 drops of stain applied by a clean medicine dropper.

3. Then add to the stain on the smear 8 to 10 drops of distilled water from a medicine dropper.

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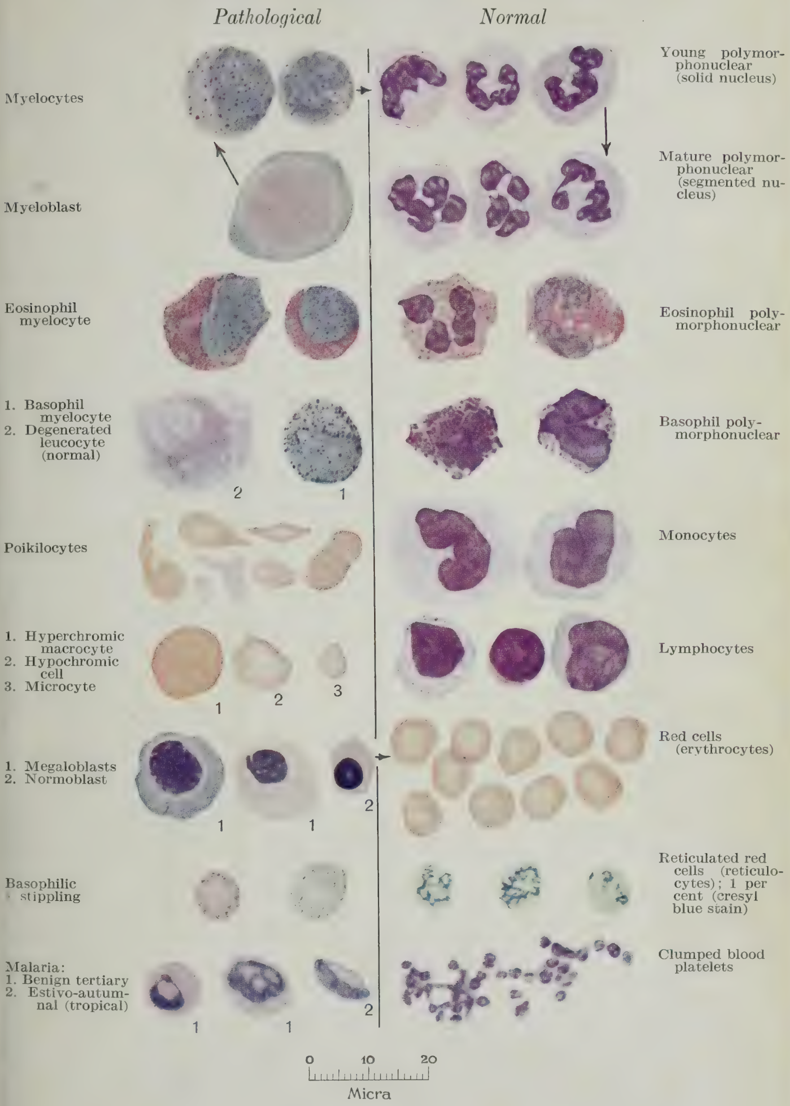
[1] I have obtained good results with stains from the National Aniline and Chemical Company of New York.

BLOOD CELLS × 1000 (1–1 mm.) WRIGHT'S STAIN



Pathological
Normal
Myelocytes
Myeloblast
Eosinophil myelocyte
1. Basophil myelocyte
2. Degenerated leucocyte (normal)
Poikilocytes
1. Hyperchromic macrocyte
2. Hypochromic cell
3. Microcyte
1. Megaloblasts
2. Normoblast
Basophilic stippling
Malaria:
1. Benign tertiary
2. Estivo-autumnal (tropical)
Young polymorphonuclear (solid nucleus)
Mature polymorphonuclear (segmented nucleus)
Eosinophil polymorphonuclear
Basophil polymorphonuclear
Monocytes
Lymphocytes
Red cells (erythrocytes)
Reticulated red cells (reticulocytes); 1 per cent (cresyl blue stain)
Clumped blood platelets
2
1
1
2
3
1
1
2
1
1
2
0
10
20
Micra

4. After three to five minutes flood the slide with an excess of distilled water so as to float the scum off the blood film.

5. Blot, dry and mount with Canada balsam under a cover-glass.

Causes of Poor Results.—(1) Precipitation on slide, (*a*) evaporation, (*b*) scum. (2) Lack of staining, (*a*) red cells, (*b*) nuclei of white cells.

1. (*a*) A precipitate of stain on the film results from evaporation of the alcohol so that it cannot hold the stain in solution. This may be overcome (1) by adding 5 to 10 per cent more of absolute methyl alcohol to the bottle of stain. (2) By using a larger pool of stain which will not evaporate so quickly. (3) By keeping air currents away from the film. (4) By covering the slide with a watch-glass or other cover. (5) The larger flakes of precipitate are the result of the sheen or scum settling when the mixed stain and water is drained off instead of flooded off. If this occurs it cannot be washed off the film by any means.

2. Lack of proper staining may be caused by not allowing sufficient time for the stain to act. When a stain is not of good quality twice the usual staining time may be allowed but if this does not produce good results the stain should be replaced by a fresh one. (*a*) If the red cells appear greenish instead of pink the stain may be of too recent preparation or the glassware or distilled water may be alkaline. Usually under these conditions the nuclei of the white cells are very well stained although the erythrocytes and the granules of the eosinophiles may be greenish. This may be corrected by adding 1 drop of decinormal hydrochloric acid or other weak acid to 100 cc. of distilled water and using this in place of the ordinary distilled water.

(*b*) If the red cells are buff or light pink and the nuclei of the white cells faint or unstained the fault is due to acid in the stain, on the slide or in the distilled water. The distilled water should be rendered slightly alkaline by a drop of decinormal sodium hydroxide or a drop of weak bicarbonate of soda. If this does not succeed the fault is in the stain and it will have to be discarded.

(*C*) EXAMINATION OF STAINED BLOOD SMEAR.—CAUTION.—Use the oil immersion lens or if the high dry lens is used for rapid differential leucocyte counts there should always be a cover-glass on the film with balsam, oil or water under it.

Examine the stained film systematically, first the red cells, then the white cells and lastly platelets. Regarding the red cells decide in the following order on the absence or presence, also the degree of:

1. **Achromia.**—Achromia is an unusual paleness in the center of some or all of the cells. In normal blood some of the cells have paler centers than others. Only after training the eye to recognize the degree of paleness of the cell centers in normal blood can the increased paleness of achromia be correctly diagnosed. If on examining a smear there be doubt on this point compare with a normal smear. The degree of pallor varies from slight in mild cases of

anemia to severe where the pessary cells with only a narrow ring of cytoplasm with a large clear center are present in severe secondary hemorrhagic anemias. The larger the central pale area of the red cells the less the percentage of hemoglobin. In pernicious anemia only the small cells are achromic, the large cells being hyperchromic. One can recognize this condition quite definitely in a stained smear. For this examination the intensity of the stain should be the same on all films or if it is not, allowance should be made for the difference between faintly stained and intensely stained films.

2. **Poikilocytosis.**—Poikilocytosis is a deviation from the circular outline. It should be classified as (1) slight, when there are only a few ovoid forms, (2) moderate, when there are definite oval and pear-shaped forms, and (3) marked, when there are grotesque distortions. Without practice one may confuse the true pathological poikilocytosis with the distortion of the cells which may occur in the spreading of a blood smear. The true pathological one has oval and pear-shaped cells and the long axis of the cells may be in any direction. The spread of the smear may cause some cells to be elongated in one direction only or they may be flattened against each other.

The cell with the irregular fuzzy margin is due to poor fixation of the film. The crenated cell is due to some foreign substance on the slide or an electrical charge resulting from rubbing the slide with silk or cloth. Diffuse stain between the cells is due to insufficient washing after staining.

3. **Anisocytosis.**—Anisocytosis is an irregularity in the size of the cells and is a very important observation. *Stated in terms of red cells, the smallest cell has, in health, never less than two-thirds the diameter of the largest cell. In 90 per cent of cells the diameter of the smallest is never less than four-fifths of the largest. Since in health very few cells are of the extreme diameters, if in several microscopic fields one sees a cell one-half the diameter of another there is a definite anisocytosis. When some cells have two or three times the diameter of others the anisocytosis is marked and the anemia is profound.* The cells larger than normal are called macrocytes and smaller than normal, microcytes.

In actual measurement the average normal red cell diameter is 7.5 microns. Ninety per cent of the normal cells vary within 6.5 to 8.5 microns and the extreme range of all normal cells is 6 to 9 microns. Microcytes are 3 to 6 microns and macrocytes 9 to 12 microns.

The variation from normal size may be roughly ascertained by making a very thin smear of normal blood and after staining and drying it, making another thin smear of the pathological blood over it. Compare the intermingled normal stained cells and the abnormal unstained cells under the oil-immersion lens of the microscope.

In the differential diagnosis of anemias the number of cells with abnormal diameters is so important that a diameter measurement of 200 consecutive cells and plotting of the curve page 47 or estimation of the average cell diameter by the halo method page 48 is worth while. The latter is more easily performed.

4. **Infrequent Findings.**—*Nucleated Red Cells.*—Nucleated cells the size of normal red cells are called normoblasts. Those smaller than normal are called microblasts and larger than normal, megaloblasts. The *cytoplasm* of the normoblast and microblast is exactly the same color, as the normal red cell. The cytoplasm of the megaloblast may be paler or a grayish-blue with the Romanowsky stains.

The nucleus is a deep blue-black, round and although usually in the center it may be near the margin. Its roundness serves to distinguish it from a platelet which may rest on an erythrocyte.

5. **Basophilic Stippling.**—Basophilic stippling, polychromatophilia or polychromasia and malarial parasites can be detected by the Romanowsky or special methylene blue staining.[1] Basophilic stippling, numerous small black dots occurring in red cells, can be recognized only with the oil immersion lens and should be distinguished from a precipitate of stain which is present in granular form on *all* the cells in the field. The dots are very small, are evenly distributed or at times there are more near the margin. (See colored plate, page 41.) None occur outside the cells. Examination should not be done on slides showing precipitated stain or débris. The red cells should not overlap and the nuclei of the white cells should stain a *deep* blue. Basophilic stippling at the most occurs in only a few cells of every microscopic field and frequently one has to search several fields before finding one stippled cell. If only one stippled cell be found in 10 microscopic fields, it has diagnostic significance. The dots may be very small in which case there are usually many of them or when they are fewer they are as large as the granules in neutrophilic leucocytes. They may occur in cells that are normal in color, in achromic or in polychromatophilic cells.

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[1] Held and Goldbloom: Methylene Blue for Basophilic Stippling, Med. Clin. North America, November, 1928. On a thoroughly clean slide make a thin blood film. Fix in alcohol, preferably methyl, for five minutes. Add to film, while still wet with alcohol, Loeffler's methylene blue, diluted with water, 1 in 8, for five minutes. Wash, dry and examine under oil immersion lens. The red cells stain green and any basophilic granules a deep blue, the same color as the nuclei of the white cells. Polychromasia may be present in lead poisoning as well as basophilic granules.

It is important to note any gross changes in the cells, as stippling occasionally occurs in severe pernicious anemia, or leukemia as well as in lead poisoning.

6. **Polychromatophilia.**—Polychromatophilia is a slate-gray or bluish color which may appear in part or all of a red cell in contrast to the normal pink or buff color of the cells.

Howell-Jolly bodies are small black dots, one or two of which occur in occasional red cells. Cabot ring bodies, a faint blue circle or figure-of-eight, are rare. They occur in severe anemias and are of little diagnostic importance.

7. **Examination of Blood Film for Malarial Parasites.**—Quinine should be withheld for several days previous to the examination and the blood should be taken any time from twenty-four hours after the chill to just before the coming one is due.

It is frequently difficult to obtain organisms in chronic types, especially the malignant tertiary. A good provocative is adrenalin, 1 cc. of 1 in 1000 injected hypodermically. The blood may be taken one-half hour later. If this does not succeed a concentration method may be tried. The infected cells are lighter than the normal cells. Centrifuge 10 cc. of citrated blood until the cells are settled but not packed. Spread the top layer of red cells on a glass slide, stain and examine them. This will usually show any organisms present.

The stain is important. A stain that does not show up the nuclei of the leucocytes distinctly will not show malarial parasites. The parasites on the cells should be bluish with red dots, and sometimes black pigment. This serves to differentiate them from platelets or flakes of stain.

The main differentiating point is that the benign tertiary and quartan have large parasites compared with the malignant form. In benign tertiary the red cells are enlarged. The cytoplasm is pale and often contains fine, bright reddish granules. The parasite, plasmodium vivax, appears bluish and has large rings with irregular outline and is dotted with pigment. (See colored plate, page 41.)

In the quartan type the red cells are normal in size and the parasite, plasmodium malariæ, may assume a band form. In the estivo autumnal or malignant tertiary the red cells are normal in size and the parasites, plasmodium falciparum, in the advanced stage assumes the crescent form which is shaped like a curved sausage, somewhat longer than a red cell is wide, has a central red nucleus, surrounded by black pigment granules and may or may not be attached to a red corpuscle.

Interpretation in Malaria.—Finding of the organism and the response to quinine is proof of the disease.

Both the benign tertiary and the estivo autumnal or malignant tertiary complete their cycle, burst through the red cell and cause an elevation of temperature every forty-eight hours. The quartan type requires seventy-two hours to do this. However, mixed infections may cause a daily or irregular fever.

If the organism cannot be found and the fever does not subside under quinine the patient is likely suffering from endocarditis or a deep lying abscess, frequently in the upper abdomen, such as subphrenic or perinephric. Rarely, latent tuberculosis or syphilis which involves the liver produces a periodic chill and fever.

8. **Important Observations Other than Red Cells. The Platelets.**—The platelets stain a slight color with hematoxylin and a deep purple with the Romanowsky stains. They are from one-fifth to one-half the size of a red cell, have roundish outline and a ragged margin. They usually occur in clumps of 3 to 8 and are irregularly distributed throughout the smear. The clumping is the first step to form a mechanical plug to an injury. Some platelets appear singly and if one lies on a red cell it may resemble a malarial parasite or the nucleus of a normoblast. Their proportion is about 1 to every 25 red cells.

9. **White Blood Cells.**—The white cells are described in detail under Differential White Cell Count on page 59.

THE MEASUREMENT OF THE CELL DIAMETERS AND THE PRICE-JONES CELL DIAMETER CURVE.

PRINCIPLE.—The diameters of cells are measured by ruled lines in the eyepiece of the microscope and the results of 200 cells noted. The results when plotted in a chart are typical for normal blood, secondary or pernicious anemias. It is a quantitative measure of anisocytosis.

Apparatus.—1. A thin film of stained blood.

2. Microscope and hemocytometer.

3. Micrometer eyepiece in which one division equals a micron under the oil immersion lens. Compare the divisions in the eyepiece with the lines on a hemocytometer adjusting the tube length of the microscope so that 50 divisions in the micrometer eyepiece cover a small square on the hemocytometer which is just 50 microns across.

The Count.—Place the blood film under the oil immersion lens with a good light and measure 100 consecutive round cells recording the number of cells as outlined below. Then move to another part of the film and record the diameters of another 100 consecutive cells. Do not measure any cell that is not round.

Plot the diameter of each one in a tally manner as follows:

Cells Counted of Each Diameter.

Diameter.		Total.	In 200
3.0	1	1	1
3.5	1	1	1
4.0	1	1	1
4.5	1	1	1
5.0	𝍸	5	4
5.5	𝍸	6	5
6.0	𝍸 𝍸 𝍸	15	11
6.5	𝍸 𝍸 𝍸 𝍸	20	15
7.0	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸	30	23
7.5	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸	60	46
8.0	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸	55	42
8.5	𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸 𝍸	45	35
9.0	𝍸 𝍸 𝍸	15	11
9.5	11	2	1
10.0	1	1	1
10.5	1	1	1
11.0	1	1	1
11.5			
12.0			
12.5			
13.0			
13.5			
14.0			
14.5			
15.0			
		260	200

This estimation can be done much more rapidly if an assistant puts down the diameters and also keeps a record of when the 200 cells are counted. In case more are counted, total the cells. If this be 260 and there are 55 cells 8 microns, in 200 cells there would be $\frac{200}{260}$ of 55 or 42 cells 8 microns. So the total number of each diameter must be reduced before plotting the graph.

Interpretation of Cell Diameter Measurements and Price-Jones Chart.—The curve of normal blood is uniformly a very acute angle with slightly outcurved bases. In anemias the angle becomes less acute, the curve at the base is very great and very irregular, due to the increase in abnormal sized cells.

Curves of various anemias are typical. In pernicious anemias the peak is always to the right of the normal peak and lower than it. There are two exceptions to this. (1) In pernicious anemias where the red cell count is a million or less the cell diameter curve is very flat and extends far to the left on account of the very small forms. Here the average cell diameter is less than normal due to the many small forms. (2) Treatment of pernicious anemia with liver diet produces a normal curve or one like secondary anemia.

There is an important group of diseases characterized by a macrocytosis that can be differentiated from pernicious anemia because they have not over 15 per cent of cells over 8.5 microns. They are hemolytic anemia of lead, radio-active substances, nitro-benzine, benzine and pyrogallol, Jaksch's anemia, anemias associated with tumors of bone, leukemia, severe malaria, hypothyroidism and a few patients with cancer of the stomach. In congenital hemolytic icterus the average diameter of the red cells is 1 micron less than normal. In hemorrhagic anemias the peak is always to the left and lower than the normal peak.

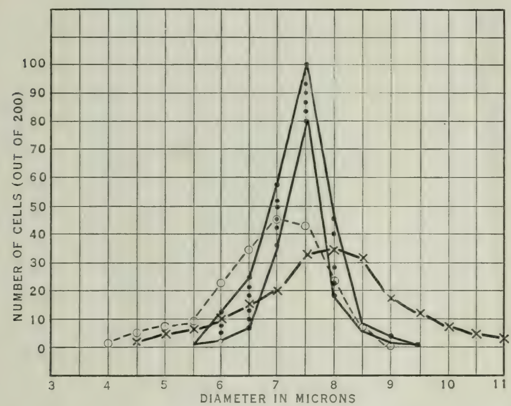

FIG. 10.—*Red Cell Diameter Graph.* Modified from Price-Jones by recording in half microns, the cell diameters, by means of a micrometer eyepiece. Solid lines represent the maximum and minim normal limits; 0--0--0 hemorrhagic anemia; x–x–x–x Addison's anemia.

In actual measurement the average cell diameter is 7.5 microns, and out of 200 red cells in health over 90 are 7.5. The total range is 6 to 9 microns. In disease it may vary from 3 to 12 microns.

The Price-Jones curve is more time consuming than the halo test.

THE HALO METHOD FOR THE DETERMINATION OF THE AVERAGE CELL DIAMETER.

PRINCIPLE.—Parallel light rays passing through a grating of red cells in front of a convex lens produce, at the focal distance of the lens, circular concentric spectrums, the size and character of which depend on the size and shape of the cells. By this means the average diameter of over a million cells may be measured.[1]

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[1] Pijper, H.: Lancet, 1924, **207**, 367.

Apparatus.—A thinly spread unstained blood smear.[1]

A kodak taking post-card-size pictures. Smaller cameras will give proportionally smaller halos.[2]

A sheet of tissue paper ruled in 5-mm. spaces and 3 inches wide to fit under the rollers at the back of the camera. Artist's copying paper serves better than tissue paper.

Sunlight or light from a 500-watt lamp.

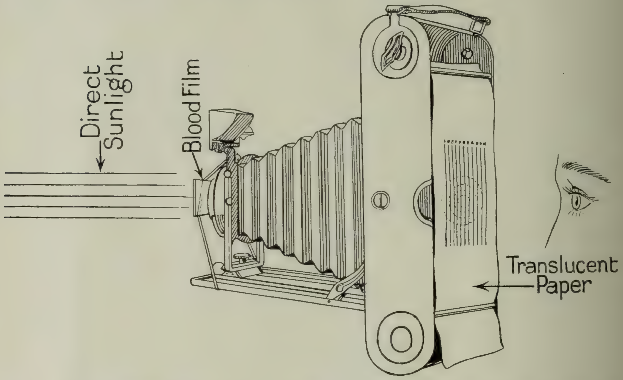

FIG. 11.—An ordinary post-card-size Kodak arranged for measuring cell diameters by the halo method.

The Test.—Remove the back of the camera and pass the sheet of ruled copying paper under the rolls to hold it in place. Open the diaphragm to its widest and also open the shutter. Draw the camera out until the blood smear is 20 cm. (8 inches) from the transparent paper in the back. Place the thinly spread part of the smear in front of the lens and hold it in place by a rubber band. If the camera be now pointed at the sun or other strong light, a halo of rainbow colors appears on the copying paper in the back of the camera. The diameter of the internal orange ring is inversely proportional to the diameter of the cells. With normal blood it is 30 to 35 mm. provided the camera be extended to 8 inches. The halos are very bright and clear-cut with several secondary ones outside.

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[1] The blood smear should be so made that the cells do not overlap or are not thinly scattered on the slide. The ordinary thinly spread film where the cells have their edges almost touching is satisfactory. Where the blood count is very low it will be necessary to make thicker smears. A glance under the low power of the microscope will determine whether or not a doubtful smear is satisfactory for the halo examination.

[2] Each camera should be standardized by extending it to a definite length and noting the exact diameter of the inner orange halo when a normal blood smear is in front of the lens. With a post-card-size camera extended so that the blood smear is 20 cm. (8 inches) from the paper in the back, the inner orange halo has a diameter of 31 to 33 mm when normal blood is used. With a smear of advanced pernicious anemia the internal orange halo has a diameter of 26 mm. The camera should always be drawn out to exactly the same length for each estimation. If a $3\frac{1}{4}$ by $4\frac{1}{4}$ size kodak is used the halo is proportionally smaller and sharper in outline. One may standardize it by finding the diameter of the internal orange halo when the camera is drawn out to a definite length and a normal blood smear used.

In pernicious and other anemias with a preponderance of macrocytes giving a larger average cell diameter the halo will be smaller than normal. The width of the internal orange ring will be from 26 to 30 mm. because the size of the halo varies inversely to the average size of the cells. Because the cells vary greatly in size the halo has an indistinct outline with the secondary halos almost imperceptible. In secondary anemias and even in congenital hemolytic jaundice where the average cell diameter is less than normal the halo is of about the same size as with normal blood but it is indistinct on account of the varying size of the cells. Thus by comparison with the halo from normal blood one can detect any abnormality in the size or outline of the cells by indistinctness of the halo and also any macrocytic tendency by a halo of small size.

Comparison of the Cell-diameter Curve of Price-Jones and the Average Diameter Estimation by the Halo Method.—While the estimation of the halo requires but a minute the cell diameter count requires from a one-half to one hour of tedious work.

The halo estimates quite accurately the average diameter of about a million cells. By it one can find out a macrocytosis with certainty. The lack of clear outline and absence of secondary color rings is the only means of estimating variation in size and outline of the cells. This cannot be expressed in a figure but if a permanent record is desired a photograph may be taken by replacing the transparent paper at the back with a photographic film and taking a picture with the blood smear immediately before the lens.

As most of the irregularly-shaped cells are small the method here outlined for cell diameter counts omits them and so tends to show a macrocytosis when there are as many cells smaller than normal as there are larger than normal. Taking the average of the least and greatest diameter of oval cells is too laborious and it is not even accurate for irregularly-shaped cells.

Interpretation in Detail of Red Cell Abnormalities.—Attaching too much importance to an elaborate examination of a small drop of blood, a fault all too common at present, will lead to frequent errors in diagnosis and prognosis. Frequently such reports are accepted as from an oracle while the whole disease-ridden body is not carefully investigated. Any blood-finding should be regarded as a special physical sign. Some are of great importance, others of slight importance.

Achromia results from a hemoglobin lack in the cell. When definitely present in many cells and there is little or no anisocytosis, it is a valuable sign of secondary anemia of the hemorrhagic or chlorotic type. Achromia is marked in most of the cells and the anisocytosis is slight when anemia is severe.

In pernicious anemia only the small cells are achromic. The large ones are usually hyperchromic. This is true to a less extent of the other chronic types of anemias which have macrocytes and which are mentioned under anisocytosis.

Poikilocytosis or Irregularity in Size.—One should always make sure that this abnormality is not due to the making of the smear. It is most marked in pernicious anemia where the cells may take the oval, pear or dumbbell shape. It is slight in moderate degrees of a secondary anemias where the irregular forms are mostly oval.

In rare instances a congenital poikilocytosis has been found in all the members of the same family.

Anisocytosis.—As the smear is the most important single examination of the blood cells so is the observation of anisocytosis or irregularity in size the most important point in the blood smear. Cell diameters vary but little in health and the severity of a chronic anemia is directly proportional to the degree the cell diameters vary from normal. The greatest variation occurs in severe pernicious anemia, where there are macrocytes, cells larger than the normal and so very full of hemoglobin that they show no pallor at all in the center. This condition also occurs to a lesser degree in anemias due to hemolytic agents. For further details see Interpretation of Price-Jones Curve of Cell Diameter, page 47 and of the Halo Test, page 48.

Microcytes also are present in all these conditions, more frequently in the severe types. They usually have very pale centers and may be irregular in shape. They are about the only abnormally-sized cells in the chlorotic type of secondary anemias.

Basophilic Stippling is the most reliable sign of *early* lead poisoning. Stippling varies from day to day and occasionally may be absent so that examination should be made on three or four consecutive days before excluding lead poisoning where it is suspected. Frequently stippled cells cannot be found at all in the late stages of the disease.

A few stippled cells occasionally appear in other chronic diseases such as pernicious anemia, leukemia, malaria and acute infections in infancy but in these the other blood changes are very gross and make the distinction easy. Only when the red cell count is below 2 million in pernicious anemia and the cells very hyperchromic and distorted does one see stippling. The leukemia changes are also very gross before stippling occurs. Only a very occasional cell is affected. In lead poisoning the blood count is only slightly lessened and never below 3 million. The red cells are regular in size, many are achromic and some of these usually contain basophilic granules. In most cases of early lead poisoning one rarely examines more than five to six oil-immersion fields before seeing stippled cells. They are never as frequent as this in other diseases so the occurrence of even over one definitely stippled cells per 100,000 or more than one in every 5 or 6 microscopic fields without other gross blood changes such as malaria, leukemia or pernicious anemia is as pathognomonic of lead poisoning as a positive Wassermann reaction is of syphilis. Exposure to lead is very general in cities, even street dust may contain it. Leake[1] has shown that the air in most factories contains traces of lead. Four per cent of normal persons in varied industries showed lead in their excreta. In garages there was $\frac{1}{3}$ milligram of lead per 10 cubic meters of air and the settled inorganic dust on benches and floors contained 1 per cent of lead. While contact with moderately large amounts is necessary to produce lead poisoning, the tolerance varies so much that stippling and symptoms are more important than quantitative lead estimations in the excreta and urine. From observations at the lead mines in British Columbia, Dr. J. F. Haszard is of the opinion that symptoms of lead poisoning occur more readily in patients whose general health is impaired by focal infections such as septic teeth or tonsils. The respiratory tract is the main port of entry for lead. Parathyroid extract has recently been tried as a remedy.[2]

Nucleated Red Cells are premature cells called out from the blood-forming capillary which extends between two blood sinusoids in the bone-marrow in an effort to replace erythrocytes which have been destroyed. They are most abundantly found in myelogenous leukemia a condition always accompanied by anemia. They come out in very acute severe secondary anemias and at times in pernicious anemias. The large nucleated cell, the megaloblast, is found in the peripheral blood more often in severe pernicious anemia than in other anemias. Physical activity aids the migration of nucleated cells into the blood stream of an anemic patient. Nucleated cells are found more frequently in ambulant patients than in bed patients.

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[1] Leake: Jour. Am. Med. Assn., 1927, **89**, 1105.

[2] Hunter and Aub: Lead Studies, Quart. Jour. Med., 1927, **78**, 123.

Polychromatophilia is the grayish-blue coloring occurring with the Romanowsky stains. It is found in severe anemias, such as pernicious, lead or other hemolytic types of anemia and is a sign of rapid blood regeneration.

Platelets.—A full interpretation of diminution in the number of platelets will be found on page 74 and of any white cell change on page 62.

RETICULATED RED CELLS (RETICULOCYTES).

PRINCIPLE.—Immersion of fresh, wet blood in a vital stain shows in premature red cells, reticular lines which are not apparent when the dried smear is stained. This is a means of estimating the rate of cell reproduction.

Simple Slide Method.—*Apparatus.*—Glass slides absolutely clean.

Coverslips 22 mm. square, absolutely clean.

Microscope.

One-ounce dropping bottle containing 0.5 per cent brilliant cresyl blue dissolved in absolute alcohol.[1]

OUTLINE OF TECHNIQUE.—1. Spread a thin film of brilliant cresyl blue on a slide. Allow it to dry.

2. Touch a coverslip to a small drop of blood and let it fall on the stained area.

3. After three minutes count under the oil-immersion lens the percentage of reticulocytes in 1000 cells.

Technique in Detail.—After cleaning the skin, puncture either the ear lobe or finger. Wipe away the first drop with dry absorbent. When a small drop, the size of a pin-head appears, touch an absolutely clean cover-glass to it. Then lay the cover-glass over the stained portion of the slide so that the fresh drop of blood spreads out between them. The stain diffuses into the blood in several minutes.

Examine under the oil-immersion lens a portion of the film where the cells are discrete.

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[1] One drop of 0.5 per cent brilliant cresyl blue dissolved in absolute alcohol is placed at one end of a glass slide and, using the end of another slide, the drop is spread out over 1 square inch of the slide in the same manner as a blood smear. The alcohol evaporates immediately, leaving a very thin film of stain at its margin. A number of slides may be so prepared and kept for use.

(Caution.—Although this procedure is extremely simple there are many possibilities of error. The stain may be so thick that granules are present and if they are under blood cells, may simulate reticulocytes. This artefact may be ruled out by observing the granular masses between the cells as well as under them. If the stain is too sparse, reticulocytes may go unstained. The stain is usually sufficient for the reticulocytes if white cells, especially their cytoplasmic granules are well-stained.

If the blood does not spread out well the cells remain heaped on each and cannot be examined. Slight pressure on the cover-glass at the time of making the smear will usually leave several hundred discrete cells in the center. Heavy pressure will distort the cells. There are too many cells in the oil-immersion field to count with ease. A paper diaphragm with a small square hole cut in it and placed in the eyepiece will give a sufficiently small area in which to count the cells.)

Appearance of the Cells.—The red cells are a yellowish-green and when reticulated cells are present, they appear normal or achromic with short, deep-blue lines interwoven in a skein fashion, sometimes in the center, sometimes like a wreath just inside the margin. (See colored plate, page 41.) The white cells stain a purplish-blue.

The Count.—Count the total number of cells in a field and also the number of cells containing any reticulation. Move the slide to other fields containing discrete cells until the reticulocytes in 1000 cells are counted. Calculate the percentage of reticulocytes.

It is necessary to examine 1000 red cells for reticulation to obtain a satisfactory average. If frequent counts are required the estimation may be facilitated when the blood spread is moderately even by counting the total number of cells in 3 or 4 fields and taking their average. Then count only the reticulocytes in each field until enough fields are examined to make the total of 1000 cells. Calculate the percentage that is reticulated.

Immersion Method.—This method, although requiring more preparation than the slide method, insures that every cell is completely saturated with the stain.

Apparatus.—The Stain.—1. 1-ounce dropping bottle containing 1 per cent aqueous brilliant cresyl blue.

2. Four-ounce bottle containing aqueous solution of 0.9 per cent sodium chloride and 0.5 per cent potassium oxalate.

The Test.—Immediately before use, to 10 cc. of the oxalated saline solution add 1 cc. of the aqueous brilliant cresyl blue. Filter, placing the filtrate, in a clean centrifuge tube.

Make a deep incision in the finger or ear lobe and allow 2 or 3 drops of blood to fall into the stain. Invert the tube to mix. Let stand fifteen minutes or more. Centrifuge for a minute; then pour off the supernatant fluid and shake up the drop remaining with the sediment. By means of a pipet place a drop of the sediment at one end of a glass slide and spread it out in the same manner as an ordinary blood smear, using a cover-glass or the end of a glass slide as a spreader. Dry the smear.

Examination.—Under the oil-immersion lens, examine 1000 cells and calculate the percentage of reticulocytes as outlined on page 53.

Interpretation.—An increased reticulocyte count is probably the earliest indication of accelerated red cell reproduction. This rapid cell formation leaves imperfections in the cell stroma and when vitally stained they are seen as deep-colored broken lines.

Under ordinary conditions there is less than 1 per cent of reticulated cells in normal blood. When there is a great demand for red cells either due to loss in hemorrhage or destruction by infection, more reticulated cells appear. At the beginning of a remission in pernicious anemia or in an important rise of the red cell count the reticulocytes form over 5 per cent. This percentage gradually falls below 1 per cent as the increase is completed.

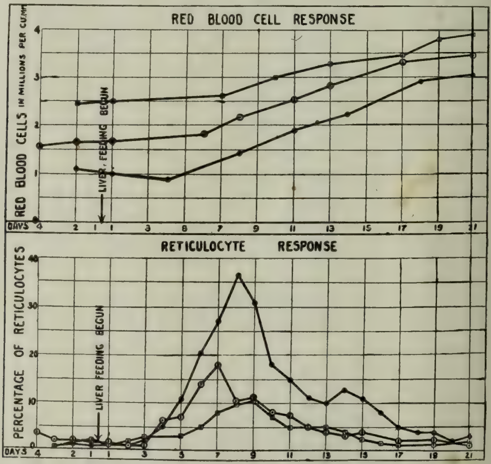

FIG. 12.—The reticulocyte response in 3 pernicious anemia patients on liver diet. The lower the initial red cell count the higher the reticulocyte response which occurs at the beginning of the total cell increase. (Minot, Murphy and Stetson: Am. Jour. Med. Sci., 1928, **175**, 581.)

A compensated anemia is attended by over 3 per cent of reticulocytes. It occurs where the cell reproduction activity of the bone-marrow keeps pace with the blood loss thus giving a normal total red cell count.

In actual anemias the percentage of reticulated red cells ranges from 0 to 20 per cent, depending on the activity of the bone-marrow. The reticulocyte increase takes place during the first two weeks of the call for new blood cells. After the first two weeks when the most urgent need is met, the reticulocyte percentage goes below 3 per cent although the hemoglobin percentage and red cell count, which may still be only half their normal values, continue to improve.

Acute hemorrhage in a healthy individual is followed by an increase of reticulated red cells from 10 to 25 per cent during the first two weeks after the hemorrhage if the patient is on an appropriate diet. A month after the hemorrhage although the patient has a moderate anemia and continues to improve there may be no reticulocytes present.

In anemia from chronic hemorrhage the percentage of reticulated cells will range from 1 to 5 and it is a good indicator of the activity of cell reproduction.

In toxic anemias from infections or chemicals the reticulated percentage is usually low because the toxic agent has a damaging effect on the bone-marrow.

Hemolytic anemia shows an increase of from 10 to 40 per cent of reticulated red cells, the greatest number occurring during the attacks of jaundice.

Prior to diet treatment of *Addison's anemia* the reticulocytes were less than 2 per cent except during the early stage of remissions when many reticulocytes can be seen. Murphy reports a great increase in reticulocytes following liver diet. An increase starts on the third day of the diet and reaches its highest point, 5 to 20 per cent, in seven days. It returns to normal after the second week while the hemoglobin and red cell count continue to increase. During this time the icterus index decreases from readings of over 15 down to normal in three to four weeks, signifying a lessened hemolysis of the imperfectly formed cells. This would point to the liver diet (cooked liver $\frac{1}{4}$ to $\frac{1}{2}$ pounds, muscle meat $\frac{1}{4}$ pound, abundance of green vegetables and fruit daily) supplying a substance which enabled the bone-marrow to manufacture normal red cells very rapidly until the cellular deficiency of the blood was made up.

In aplastic anemia there are no reticulocytes in the blood because the exhausted bone-marrow cannot supply new cells. It is always worth while to apply the therapeutic test of liver diet if a cause cannot be found. After four or five days examine for reticulocytes. Sometimes they appear in Addison's anemia of the aplastic type. More frequently none are seen and so one cannot expect improvement. Blood transfusion as a temporary remedy may be considered.

OUTLINE OF WHITE CELL DIFFERENTIAL COUNT.

Making the Blood Film.—Place a small drop of blood near one end of a glass slide. Spread it out with the end of a cover-glass or other spreader by making the blood follow it. The smear should not be too thin.

Routine Stain.—Alcoholic eosin, one to two minutes. Wash. Hematoxylin two to five minutes. Wash.

Special Stains.—Wright's, or Leishman's. Stain for one minute. Add distilled water three to five minutes. Flood the slide and wash off with distilled water.

At first differential counts should be done under the oil-immersion lens. Later the high dry lens may be used provided the film be mounted under a cover-glass.

The Count.—Count about 100 white cells at 1, 2 and 3. Record them by the tally system and calculate the percentage of each variety.

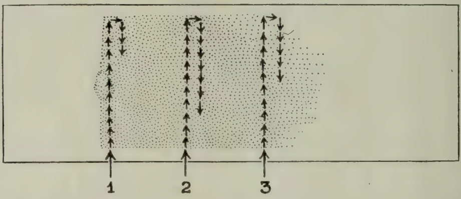

Fig. 13.—For a differential count move the slide to count 100 cells across the film at 1. Then make a similar count at 2 and 3 and take the average.

	Normal per cent.	Tally count.	Per cent.
1. Lymphocytes	20 to 30	~~1111~~	
2. Monocytes	5 to 10	11	
3. Eosinophiles, polymorphonuclear	2 to 5	1	
4. Neutrophiles			
polymorphonuclear: unsegmented 5 to 10 segmented 55 to 70	60 to 75	~~1111~~	~~1111~~
5. Myelocytes	0		
6. Undetermined types (usually number 2, 5 or 6).			

The basophile and myeloblast are omitted from the list because they are rare cells and of no diagnostic importance.

If 300 cells are counted the error is less than 3 per cent.

If only 100 are counted the error is over 10 per cent. (Beacom.)

Interpretation of the Total and Differential Leucocyte Count in Brief.—Infections resulting from cocci of any variety produced a leucocytosis which is due to an increase in the polymorphonuclear neutrophile cells.

Over 85 per cent of polymorphonuclears points to pus formation; 90 per cent to a very severe infection. The resistance is good if the total leucocytes are increased proportionally and poor if they are not increased. There should be

15,000 total with 80 per cent polymorphonuclears.
20,000 total with 85 per cent polymorphonuclears.
25,000 total with 90 per cent polymorphonuclears.

The percentage that the young unsegmented band-shaped polymorphonuclears is of the total white cells is a more valuable index. If taken at four or eight-hour intervals, it is a better prognostic guide than the temperature chart.

10 to 15 per cent indicates a mild infection.
15 to 30 per cent indicates a moderately severe infection.
30 to 40 per cent indicates a very severe infection.
Over 50 per cent indicates a fatal infection.

The absence of eosinophiles is an unfavorable sign. Their presence is favorable.

Infections resulting from bacilli of any variety produce a mild lymphocytosis. Any infection, especially tuberculosis, involving lymph glands, will produce a lymphocytosis. Whooping cough produces a leucocytosis of 20,000 to 30,000 ot which 60 per cent are lymphocytes. An increase in the lymphocytic percentage without total increase of leucocytes occurs in influenza, typhoid (monocytes), exophthalmic goiter and malaria. The presence of many myelocytes or lymphocytes in the blood of a patient who has little or no fever points to leukemia.

Technique in Detail of Differential White Cell Counting.—First examine enough white cells under the oil-immersion lens to become thoroughly acquainted with their appearance. After this the cells may be rapidly examined under the high dry lens provided the film is mounted under a cover-glass with balsam, cedar oil or even water. The field is so very small with the oil-immersion lens that it required much time to observe several hundred white cells. In fact the best bird's eye view of the white cells, their position and their frequency is obtained under the low-power lens. One can even distinguish lymphocytes from polymorphonuclears and eosinophiles under the low power.

To those who are not at daily practise in differential leucocyte counting, the long time occupied by the examination makes them neglect this valuable procedure. The time occupied is not so long as is generally thought. One-half hour should do, using the high dry lens. The thickness of the blood film is important. If it be too thin much time will be taken to find 300 white cells. If the smear be too thick it is difficult to recognize the various types of cells present. A film so thin that one can hardly see the spread is too thin for differential counting. One that before it is stained is opaque and red is too thick. The ideal is where the red cells slightly overlap each other as viewed under the microscope. It will save much time to make a new smear that is suitable rather than try to eke out a count on one that is too thin or too thick. Even satisfactory films are thicker near the beginning of the smear than they are near the end. The white cells as a whole, especially the polymorphonuclears, are larger and lighter than the red cells so if they are not intimately mixed with the red cells they tend to float out. In the thicker areas of the spread one finds the cells evenly distributed but in the thinner areas most of the white cells are at the margin and very end. Any error which might ordinarily occur from this irregular distribution is best taken care of by Dr. Beacom's suggestion that 100 cells be counted across each end of the smear as well as across the center.

Two of the most important items are the moving of the slide and the recording of the types. For moving the slide a mechanical stage is the best possible arrangement. One can examine the smear in an orderly manner and move the slide about rapidly. Mechanical stages are now built into the microscopes at a moderate extra cost. One can do a differential count without it, however, by placing the left thumb firmly on the stage at the left corner of the slide and rolling it gently against the slide. One may thus move the slide ever so little at a time across the field of the microscope objective lens.

Recording the types of cells is perhaps the most irksome part of the count. The usual method is to write down the names of the cells and make a stroke after each cell counted, every fifth stroke crossing the other four. When a large number is tallied they are totalled and if 300 or over the percentage of each may be recorded as follows; suppose out of a total of 327 cells there were 95 lymphocytes, the percentage of lymphocytes would be $\frac{95}{327}$ of 100 or 29 per cent. The percentage of each type is similarly calculated.

There are better methods however. Marking down only the rare cells such as the eosinophiles and monocytes and keeping a mental record of the lymphocytes and polymorphonuclears until 30 or 40 are counted, is perhaps the most rapid method. If this be found difficult Todd's suggestion of using beans to record the counts is a good one. Have three lots of 100 beans and have tumblers or slide-box compartments labelled with the different types of white cells, the nearest compartments being for the polymorphonuclears and lymphocytes which are most common. In a given field if there be 4 polymorphonuclears, 2 lymphocytes and 1 monocyte, into the containers marked polymorphonuclears, lymphocytes and monocytes one puts respectively 4 beans, 2 beans and 1 bean. When 100 beans are used move to the center of the spread and count another 100 cells and then to the end of the spread and count the last 100 cells. The number of beans in any container divided by 3 represents the percentage of that type of cell.

Types of Cells.—1. **Lymphocytes.**—The smallest of these cells are about the same size as a red cell and have a very deep-staining blue-black nucleus. It almost fills the cell, leaving a narrow ring of light-blue cytoplasm which at times can be seen only at one side. The larger types are about twice the size of the smaller. They have a nucleus which is slightly larger and takes on a less dense stain. The clear, blue cytoplasm forms a ring equal in width and at times greater than the nucleus. The largest of these cells which are up to 12 microns in diameter are equal in size to the polymorphonuclears. This point serves to differentiate them from the monocytes which are larger. Lymphocytes of all sizes between these extremes are encountered in blood smears. Formerly the large and small lymphocytes were classed separately but there is not enough diagnostic value to justify two groups. It has been suggested that the large lymphocytes are the young forms, which as they become mature, lose their cytoplasm and are consequently smaller.

2. **Monocytes.**—These cells have a diameter which varies from 12 microns, the size of a polymorphonuclear up to and over 20 microns. The nucleus is usually round but may be deeply indented at one side. Usually it is not as wide as the ring of cytoplasm. With the Romanowsky stains a few granules may appear in the blue cytoplasm near the nucleus. The nuclei of these cells may be faintly stained and the cell outline irregular. This group of cells contains several sub-varieties such as the large mononuclear, the transitional cell and the endotheliocyte. The large mononuclear is probably derived from lymphoid reticulo-endothelium. They do not contain granules but ingest foreign particles readily. The transitional cell of Naegeli sometimes contains a few granules around the

nucleus and may be of myeloid origin or merely a degenerated form of the large mononuclear. Its most outstanding characteristic is its deeply indented nucleus but its bluish cytoplasm and the scarcity of granules in it serve to distinguish it from the polymorphonuclear neutrophile. The endotheliocyte is a mononuclear phagocyte which is derived from the blood vascular endothelium when it is stimulated by foreign material in the blood. In appearance it has much in common with the sub-varieties such as the large mononuclear, the transitional cell and the endotheliocyte.

3. **Eosinophile Polymorphonuclears.**—These cells are very striking in that their cytoplasm contains many *coarse* granules which stain very deeply with the eosin. The cell outline is round and has a diameter of from 10 to 12 microns. Frequently the cell is ruptured and the granules strewn loosely about the nucleus. The nucleus is a deep blue and may be either very deeply indented or segmented.

4. **Neutrophile Segmented Polymorphonuclears.**—These cells are the mature type and have their nuclear material in two, three or more masses which may be held together by fine threads of the same substance. It occupies from one-quarter to one-third of the cell substance, the rest being filled with numerous small granules which show up well with the Romanowsky stains but cannot be seen with hematoxylin. The cell has a diameter of 10 to 12 microns from one and a half to twice the diameter of a red cell and always has a circular outline.

5. **Neutrophile Polymorphonuclear Unsegmented.**—This cell differs from the segmented polymorphonuclear only in that it has a solid band form of nucleus which is usually U-shaped. Schilling subdivides this group into (1) the metamyelocyte which has a very plump nucleus and is the youngest form found in the blood stream in about 1 per cent, and (2) the cell that has a narrow-band nucleus deeply stained which is more mature than the metamyelocyte form but younger than the type with the segmented nucleus, normally 4 per cent of these cells are present. The percentage of these young cells increases with the severity of the infection. To one not in constant practice it is difficult to distinguish the young form from the staff-shaped form but one can obtain good results by grouping them together.

6. **Myelocytes.**—These cells are larger than the polymorphonuclears. They vary from 12 to 20 microns in width which is equal to from 2 to 3 diameters of the red cell. They have a large round nucleus which more than half fills the cell and may be flattened on one margin. A stain does not differentiate the nucleus from the

cytoplasm so distinctly in these cells as in the polymorphonuclears or lymphocytes. In a film stained with hematoxylin the large size of the cell and the large faint-staining nucleus differentiate them from monocytes whose nucleus is smaller. If a Romanowsky stain or peroxidase reaction has been used the cytoplasm appears filled with a varying number of granules which are distributed regularly, as many at the cell margin as near the nucleus. These granules are usually the small neutrophilic type but in leukemia many eosinophilic myelocytes appear with large red eosinophilic granules and basophilic myelocytes with blue granules. The peroxidase reaction is the last court of appeal to differentiate these cells from monocytes. Some investigators have reported at times 1 per cent myelocytes in the blood of normal people. As there is no distinct line between the myelocyte with its round or almost round nucleus and the metamyelocyte normally found in the blood with its indented nucleus, cells on the borderline may readily be put into either class.

7. **Myeloblasts.**—These cells are about the same size as the myelocytes and differ from them in having few or no granules. They are the parent cell of the myelocyte as the myelocyte is the parent cell of the polymorphonuclear. They are present in the blood only when there are large numbers of myelocytes; in advanced leukemias myelocytic cells showing all degrees of granularity will be present.

8. **Basophiles.**—These cells differ from the neutrophiles in that they are smaller, being 8 to 10 microns in diameter, they have coarse, bluish granules and the nucleus is not usually segmented. They are increased only in myelogenous leukemia.

9. **Undetermined Cells.**—It is frequently difficult to differentiate large lymphocytes, monocytes, and young polymorphonuclears that are between the myelocyte and polymorphonuclear stage. Although an occasional lymphocyte is slightly larger than a polymorphonuclear there will be fewer mistakes made if a definite division between lymphocytes and monocytes be made at the diameter of the polymorphonuclear. Other features such as the character of the nucleus or the cytoplasm are too variable and indefinite to use in classification. A few myelocytes may be present in severe infections or aleukemic types of leukemia where the myelocytes, instead of coming out into the blood stream, stuff the liver, kidneys and other organs. In severe anemias megaloblasts whose cytoplasm shows polychromatophilia may confuse the examiner.

Usually when myelocytes are present there is a marked increase in the polymorphonuclear percentage and the total leucocyte count.

Myeloblasts, the parent cell of the myelocyte, can be ruled out at once if the numerous myelocytes are not present. Myeloblasts occur only in advanced stages of leukemia. The megaloblast, a very primitive red cell comes out in the blood stream only in the presence of very severe anemia, usually with a high color index. These points although they fail at times are the most definite and practical one can use for differentiating the undetermined cell.

With the Romanowsky stains, the blue cytoplasm of the lymphocytes and monocytes is distinctive and many granules are present in the polymorphonuclears and myelocytes. These granules are very much enlarged by the peroxidase reaction. Myeloblasts need only be considered if there be many myelocytes present.

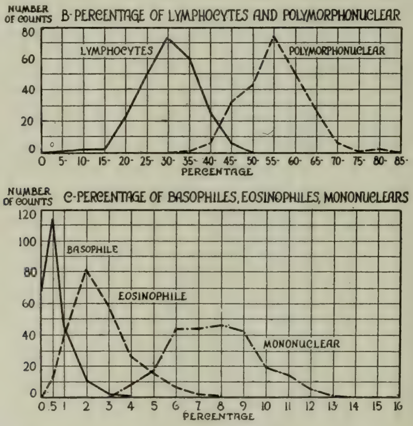

Fig. 14.—Differential leucocyte counts in normal adults showing the relative frequency as well as extent of variation of percentages. Analysis of 241 counts from 17 individuals, New York City. (Medlar: Am. Jour. Med. Sci., 1929, **177**, 72.)

Interpretation of White Cell Differential Counts in Detail.—Any increase in the total leucocyte count is usually due to an increase in the neutrophile polymorphonuclear cells, which are of myeloid origin. Infection resulting from cocci of any variety, produce an increase in the polymorphonuclears. Streptococci, pneumococci, staphylococci and meningococci are the commonest offenders. These organisms produce some of our most serious diseases such as appendicitis, pneumonia, meningitis and puerperal sepsis.

An increase in the total white cell count, wholly due to polymorphonuclears, is known as an *absolute polymorphonuclear leucocytosis.* If the infection is very severe it may use up the white cells as fast as they are made, leaving the total leucocyte count normal but the percentage of polymorphonuclears markedly increased. This state is known as a *relative polymorphonuclear leucocytosis.*

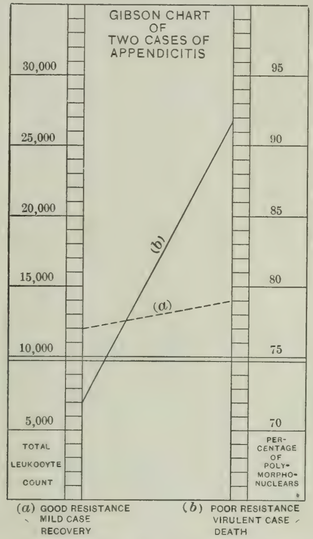

Fig. 15.—Gibson chart with blood count in 2 cases of appendicitis. Dotted line represents a mild case with prompt recovery; the continuous line, a very virulent streptococcic case with poor resistance, peritonitis and early death.

The increase in the percentage of polymorphonuclears indicates the severity of the infection, over 85 per cent pointing to pus formation. The total white cell increase indicates the resistive powers of the patient.

The Schilling count is a modification of the Arneth count which recorded the number of lobes in each polymorphonuclear cell. Arneth's work led him to believe that as the cells became more mature the number of lobes increased. Schilling maintains, and the evidence favors his view, that a bar-shaped nucleus may segment into two, three or more lobes. He divides the polymorphonuclears into four groups, (1) the myelocytes, or metamyelocytes, with round or slightly indented nuclei present in the bone-marrow but not in the blood stream, (2) the young form having a plump light-staining nucleus and normally making up 1 per cent of the total count, (3) the staff cell having a narrower dark-staining nucleus and making up 4 per cent of the total count, (4) all the segmented nuclear types which are the mature forms.

As Pons and Krumbhaar state, the essential diagnostic purpose is served and the examination simplified by subdividing neutrophile cells only into,

1. Myelocytes or metamyelocytes, very young, 0 per cent.
2. Band nuclear forms, young, 5 to 10 per cent.
3. Segmented nuclear forms, mature, 50 to 65 per cent.

The Simplified Schilling Differential Count.—Infections use up or destroy polymorphonuclears very rapidly and this loss is made up from the bone-marrow by young forms which have a non-segmented nucleus in contrast to the mature forms having a segmented nucleus.

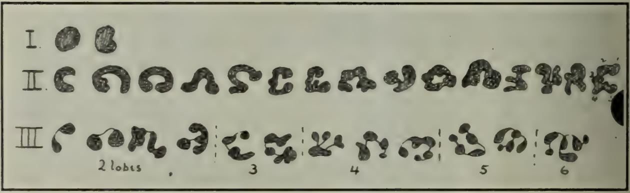

Fig. 16.—Simplified Schilling differential leucocyte count. Showing the various forms of nuclei in the 3 groups of polymorphonuclear neutrophile. First row are premature myelocytes. The second row, the unsegmented young forms. In the third row are segmented old forms with 2 to 6 lobes. The five swellings of the last nucleus of the second row are numbered to show a possible confusion with segmented forms. On account of the thick connecting bands, this is considered an unsegmented young form. (Pons and Krumbhaar, Jour. Lab. and Clin. Med., 1924, **10**, 123.)

Five per cent of young forms are present to supply the normal demands on the leucocytes but in infection the percentage is greater. This is known as a shift to the left. The extent to which this change takes place is an index of the severity of the infection.

From 10 to 15 per cent of the young forms indicates a mild but definite infection. From 15 to 30 per cent indicates that the infection is moderately severe. From 30 to 40 per cent points to a very severe infection. If over 50 per cent of young forms are present the infection is usually fatal.

TYPICAL BLOOD PICTURE OF INFECTION DUE TO STAPHYLOCOCCI, STREPTOCOCCI, PNEUMOCOCCI OR MENINGOCOCCI.

	Prognosis favorable.		Unfavorable.
	Mild.	Severe.	
White blood cells:			
Number per cubic millimeter	15,000	28,000	4000
Differential count:			
Lymphocytes, per cent	18	10	8
Monocytes, per cent	7	5	3
Eosinophile polymorphonuclears, per cent	3	0	0
Neutrophile polymorphonuclears: young, band nucleus	12	25	45
Neutrophile polymorphonuclears: mature, segmented	60	58	39
	— 72	— 83	— 84
Myelocytes, per cent	0	2	5
Undetermined	0	0	0
Platelets per cubic millimeter	500,000	200,000	100,000

A total leucocyte count of 15,000 with 15 per cent of young forms and not more than 2 per cent of eosinophiles is a picture showing a moderate degree of infection and one may arrive at a prognosis only by doing further examinations at four- to eight-hour intervals.

A leucocytosis of 20,000 to 25,000 showing 20 to 30 per cent of young forms, no eosinophiles, denotes a grave condition which may be recoverable. The prognosis is bad if in the unsegmented polymorphonuclears the number of young forms with the wide nuclei exceeds the number of band forms with the narrow deeply-staining nuclei.

An increase in the total number of leucocytes with a lessening of the eosinophiles indicates an extending infection to which considerable reaction is taking place whereas a fall in the total leucocytes with an increase in the eosinophiles indicates a recovery from the infection. A lymphocytosis may accompany the eosinophiles of recovery.

If there is a total leucocyte count over 25,000 with little or no fever and an enlarged spleen a diagnosis of myeloid leukemia should be made.

Bacillary infections do not produce an increase of the polymorphonuclear cells but are accompanied by some changes in the lymphocytes and one may classify the states as absolute or relative lymphocytosis. An example of *relative lymphocytosis* is influenza which causes a normal or decreased total leucocyte count of which the percentage of lymphocytes is frequently increased. In typhoid fever the leucocyte count is normal or decreased and there is a slight increase in the monocytes. Whooping cough produces an *absolute increase in the lymphocytes*, with a total white cell count of 15,000 or more of which 60 per cent are lymphocytes. There is an increase in the percentage of young forms of polymorphonuclears in all bacillary infections although the total polymorphonuclears are not increased.

TYPICAL BLOOD PICTURE OF BACILLARY INFECTION.

		Mild.		Severe.
White blood cells:				
Number per cubic millimeter		5,700		3,000
Differential count:				
Lymphocytes, per cent		28		40
Monocytes, per cent		12		23
Eosinophile polymorphonuclears		3		0
Neutrophile polymorphonuclears: young, band nucleus	9		18	
mature, segmented	48		17	
	—	57	—	35
Myelocytes		0		2
Undetermined		0		0
Platelets per cubic millimeter		400,000		70,000

Acute infectious diseases in which there is a monocytosis induce a lifelong immunity. Prominent examples are typhoid, whooping cough, measles, chickenpox and mumps. These diseases affect the reticulo-endothelial tissue. On the other hand endothelial monocytosis, which is often accompanied by a hemorrhagic tendency, occurs in bacterial endocarditis and at the end of septicemia.

In mixed infections, which are very frequent, the white blood picture usually mirrors the type of infection that predominates. In typhoid fever, if a septic complication such as peritonitis, arthritis or osteomyelitis due to streptococci, sets in the leucocyte picture changes from one typical of typhoid to one typical of a streptococcal infection.

Diseases due to protozoan infections such as amœbic dysentery, syphilis or malaria are usually accompanied by an increase in the monocytes. Exophthalmic goiter is usually accompanied by a general lymphoid hyperplasia throughout the body and this is reflected in the blood stream by an increased percentage of lymphocytes. Monocytosis and lymphocytosis are not definite means of diagnosis however. Any infection involving a number of lymph glands such as tuberculosis or at times new growth such as lymphosarcoma, will cause an increased percentage of lymphocytes in the blood.

Finally it is at times impossible to distinguish leukemoid blood pictures seen in bacillary infection from the leukemias. Krumbhaar has quoted cases of acute infections having myelocytes and even myeloblasts. Patients suffering from measles or pertussis have had over 75,000 leucocytes per cu.mm. over 80 per cent of which were lymphocytes. If the lymphocytic percentage is over 90 with glandular enlargement and but little fever the case is almost certainly one of lymphatic leukemia.

Rare Conditions.—Agranulocytic angina or agranulosis occurs in patients suffering from an ulcerative lesion in the mouth or throat accompanied by an extreme leukopenia in which there are few or no polymorphonuclears. There are usually eosinophiles and platelets present indicating that the whole bone-marrow is not exhausted. These states have much in common with the monocytes and the infection is due to Vincent's organisms. Glandular fever or infectious mononucleosis may be another form in which the source of a similar infection is obscure.

The Peroxidase Reaction (Sato and Sekiya).—*Reagents.*—One-half per cent copper sulphate solution is distilled water. Benzidine solution (moisten 0.02 gm. benzidine with a few drops of water. Then grind. Add 200 cc. distilled water and filter. To the filtrate add 0.25 cc. of 3 per cent hydrogen peroxide. Keep this solution in a brown bottle in the dark when not in use. It will remain good for a year although some precipitation occurs).

Safranin, 1 per cent aqueous solution.

The Stain.—To a fresh dry blood smear apply the copper sulphate solution for twenty seconds. Drain off and apply the benzidine solution for eight minutes. Drain this off and add the safranin solution for two minutes. Wash, dry and mount in cedar oil.

Examination.—Under the oil-immersion lens the peroxidase granules of myelocytes and polymorphonuclears have a deep bluish-green color while the nuclei are stained orange with the counter stain. The granules vary in number and size. The eosinophiles have large heavy granules while others are densely granular. The myelocytes may show only a few granules, the younger they are, that is the nearer to the parent myeloblasts, the fewer granules they have. The lymphocytes have no granules and the monocytes only a few near the nucleus.

The peroxidase reaction merely magnifies granules and serves to distinguish between the white cells of bone-marrow origin and those of lymphatic or reticulo-endothelial origin. The abnormal cells in myelogenous leukemia are of bone-marrow origin; those in lymphatic leukemia of lymphatic origin. All myelocytes and polymorphonuclear cells show peroxidase granules. This reaction is peculiar in that it does not show up granules in the leucocytes of patients suffering from acute encephalitis or bilateral lesions of the corpora striata of the brain. As recovery takes place in encephalitis the granules reappear so that it may be used as a prognostic sign.

The length of staining time with the benzidine solution necessary to produce a positive reaction is directly proportional to the seriousness of the prognosis. No other peroxidase methods show this lack of granules in encephalitis and one of the other simple methods may be used to demonstrate that the lack of granulation is due to this disease when it is suspected.

The Benzidine Peroxidase Reaction.—Dissolve in 10 cc. of 90 to 99 per cent alcohol a pinch of benzidine measured on the tip of a penknife. Cover a fresh smear of blood with this solution for one minute, then add an equal amount of solution made by adding 1 drop of hydrogen peroxide to 10 cc. of distilled water. Wash after three minutes. With this process the granules appear yellowish. A counter stain of methylene blue or Wright's may be added to outline the nuclei and mark the lymphocytes.

CELL VOLUME. (CORPUSCULAR VOLUME.)

PRINCIPLE.—A column of blood is drawn into a graduated tube, anticoagulant added and the tube centrifuged to pack the cells in one end. The percentage of the original blood volume that the cells pack into is the cell volume.

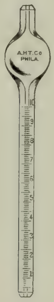

FIG. 17.—Hematocrit tube (Van Allen). For estimation of relative cell volume, using finger-tip blood.

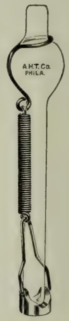

FIG. 18.—Spring sealing clip for use with hematocrit tube.

Micro Method.—*Apparatus and Reagents.*—Leucocyte pipet.[1]
Fine wire to pass through stem of pipet.
Rubber pad and heavy rubber band.
Spring lancet.

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[1] The special Van Allen pipet of sturdy build with a closely graduated stem and special metal cap should be used by anyone doing frequent estimations.

One hundred cubic centimeters of 1.4 per cent sodium oxalate. (Fresh.)
Gauze sponges and alcohol.
Electric centrifuge capable of 2000 or more revolutions per minute.

Collection of Specimen.—Obtain a large drop of blood as described for the leucocyte count. Draw blood up to the "1" mark on the leucocyte pipet, then draw up 1.4 per cent sodium oxalate to half fill the pipet bulb. Put the thick rubber pad over the tip of the leucocyte pipet stem and hold it in place by passing a rubber band over both ends of the pipet. Place the pipet point down in a centrifuge tube padding it below and on the sides with cotton. Fill another tube with water to balance it and centrifuge at high speed of 2000 revolutions per minute for over twenty minutes. Test out each centrifuge at first to determine the time necessary for complete sedimentation by centrifuging for additional ten-minute periods and noting the time when the column ceases to go lower. As a routine, centrifuge for ten minutes longer than this time because the cells in some bloods do not settle as quickly as in others. The stem of the pipet is graduated in 10 equal parts. If the column extends to midway between the fourth and fifth mark the cell volume would be 45 per cent or 45 cc. of cells per 100 cc. of whole blood. The stem of the Van Allen pipet is graduated in hundredths and readings are easily made.

Cleaning the Pipet.—The column of blood is usually solidly fixed and the easiest way of removing it is to pass up through the cell mass a small wire stilette such as is used in a 23- or 24-gauge hypodermic needle and that will readily pass through the empty stem. After this the pipet may be washed out with dilute acetic or water and finally dried by alcohol ether and a current of air drawn through it. *Normal: 40 to 50 per cent of cells or 40 to 50 cc. of cells per 100 cc. of whole blood.*

Method Using Venous Blood.[1]—This method requires 10 cc. of blood from a vein and is less liable to error than the micro-method.

Apparatus.—Glass syringe 10- to 20-cc. capacity.
Hypodermic needle, gauge 22.
Tourniquet, absorbent cotton and alcohol.
15-cc. graduated centrifuge tube.
100 cc. of 1.4 per cent sodium oxalate.
Electric centrifuge capable of 2000 or more revolutions per minute.

Obtaining the Specimen.—After placing 2 cc. of 1.4 per cent sodium oxalate solution in a 15-cc. graduated centrifuge tube, obtain 10 cc.

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[1] Hooper, Smith, Belt and Whipple: Am. Jour. Physiol., 1920, **51**, 205.

of blood from the most prominent vein at the bend of the elbow as described under the collecting of blood for chemical examination (page 197). Immediately place exactly 10 cc. into the graduated centrifuge. This will bring the total volume to 12 cc. Cover the mouth of the tube with the thumb and invert the tube slowly twice to mix the anticoagulant oxalate and the blood. Centrifuge at high speed for one-half hour and then for ten-minute intervals as described under the micro-method until no further settling of the cells occurs. The normal height of the column is 4 to 5 cc. of cells for each 10 cc. of whole blood.

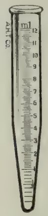

Fig. 19.—Graduated centrifuge tube for estimation of relative cell volume, using venous blood.

Normal Blood Volume in 137 *Men Aged Nineteen to Thirty Years.*

Average, 45 cc. of cells per 100 cc. of whole blood.

90 per cent of men—40 to 50 cc. of cells per 100 cc. of whole blood.

Total range—36 to 52 cc. of cells per 100 cc. of whole blood.

Normal Blood Volume in 100 *Women Aged Nineteen to Thirty Years.*[1]

Average—41 cc. of cells per 100 cc. of whole blood.

90 per cent of women—37 to 45 cc. of cells per 100 cc. of whole blood.

Total range—35 to 46 cc. of cells per 100 cc. of whole blood.

Interpretation.—The cell volume is decreased in anemia. Its decrease is a good indicator of the severity of the anemia. It may be 25 to 35 per cent of the total blood volume where the red cells are 3,000,000 and 10 to 15 per cent where the red cells are 1,000,000. In slight anemias the cell volume varies in much the same manner as the red cell count but in the severe secondary anemias of the chlorotic type the cells are smaller than normal and the cell volume is lower than the red cell count. In pernicious anemia many of the cells are larger than normal and the cell volume is much decrease although not so markedly as the red cell count. This is an important diagnostic point and it is expressed numerically by the volume index. The actual diameter of the cells when spread on a slide and 200 are measured under the microscope with an eyepiece micrometer confirms the statement about the preponderance of large cells in pernicious anemia and small cells in secondary anemia. The cell volume is increased in polycythemia.

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[1] Osgood and Hoskins: Relation Between Cell Count, Cell Volume and Hemoglobin Content of Venous Blood of Normal Young Women, Arch. Int. Med., 1927, **39**, 643.

General Remarks.—In cell-volume estimations there is less chance for subjective errors than either the hemoglobin or cell count. The time occupied in taking the blood specimen is short and other duties may be performed while the centrifuge is precipitating the cells.

The cell volume, hemoglobin and red cell count are strikingly parallel in normal individuals. In anemia they are only roughly parallel. Of the three the hemoglobin changes most rapidly; there are daily variations of 15 per cent. The cell volume is perhaps more mobile than the cell count although only slightly so.

The Volume Index.—To obtain this index, an estimation of the cell volume and a red cell count are necessary.

The volume index is:

$$\frac{\text{volume percentage red cells of normal (45)}}{\text{number percentage red cells of normal (5,000,000)}}$$

Taking 45 as the normal cell volume, estimate the patient's percentage cell volume of 45. This result is divided by the percentage blood count of the patient.

Example.—With a cell volume of 32 cc. per 100 cc. of whole blood and a red cell count of 4.1 million.

The cell volume percentage of normal is $\frac{32}{45}$ of 100 = 71.

The blood count percentage of normal is $\frac{4.1}{5}$ = 82.

The volume index is $\frac{71}{82}$ = 0.86. Normal 0.9–1.1.

Interpretation.—The volume index is a valuable aid in deciding on a questionable anemia. In pernicious anemia a volume index above 1.1 is almost a constant finding. It is frequently present in early cases before other changes occur. The color index is never greater and usually less than the volume index[1] so that a volume index above 1.1 in the blood of a patient who has no free hydrochloric acid in the gastric contents is strong evidence of pernicious anemia. In secondary anemia the volume index is normal or below normal.

The Volume and Hemoglobin Content of the Red Blood Corpuscle.[2]—The following estimations afford a more concise idea of the size and hemoglobin content of red cells than do various indexes. $\frac{\text{Vol. (cc. per 1000 cc.)}}{\text{Red blood cells (in millions)}}$ = corpuscular volume in cubic microns. Average 83.8 cubic microns (68.3–95.6). This means much the same as the volume index.

$\frac{\text{Hb. (gm. in 1000 cc.)}}{\text{Red blood cells (in millions)}}$ = corpuscular hemoglobin in 10 gm. Average 28.7, 10 gm. (24.5–34.7).

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[1] Haden: The Value of Volume Index, Jour. Am. Med. Assn., 1924, **83**, 671.

[2] Wintrobe: Am. Jour. Med. Sci., 1929, **177**, 513.

This is an absolute determination that means much the same as the color index.

$$\frac{\text{Hb. (gm. per 100 cc.)}}{\text{Vol. (cc. per 100 cc.)}} = \text{proportion of hemoglobin in a cell. Average 33.3}$$

per cent (30 per cent to 46.5 per cent).

This means much the same as the saturation index.

Haden has found an average corpuscular volume of 128 cubic microns in 20 cases of Addison's anemia. In hemorrhagic anemia the average was 71 cubic microns.

Although Addison's anemia is regarded as hyperchromic actual determination shows that the hemoglobin makes up a smaller percentage of the cell than normal. Haden found that 20 cases ranged from 16 per cent to 34.9 per cent.

Cell Hemoglobin Index.—Cell hemoglobin index or saturation index is

$$\frac{\text{Hemoglobin percentage}}{\text{Cell volume percentage of 45.}}$$

The saturation index is never greater than 1 but it is decreased more in secondary than pernicious anemia. It is not well established for diagnostic purposes.

THE AVERAGE VOLUME, COLOR AND SATURATION INDEXES OF SECONDARY AND PERNICIOUS ANEMIA CONTRASTED WITH NORMAL BLOOD. (HADEN.[1])

Red blood cells in million per cu.mm.	Percentage by volume of red blood cells.	Hemoglobin, per cent.	Volume index.	Color index.	Saturation index.	
4.74	95	95	1.00	1.00	1.00	Average 52 normal men and women.
1.71	47	44	1.41	1.29	0.92	Average 50 cases pernicious anemia.
3.40	63	55	0.94	0.81	0.86	Average 47 cases secondary anemia not due to hemorrhage.
3.57	55	44	0.77	0.62	0.80	Average 7 cases hemorrhagic anemia.

BLOOD PLATELETS.

PHYSICAL CHARACTERISTICS AND DIFFICULTIES OF EXAMINATION.—The blood platelets are roundish bodies from one-fifth to one-half the diameter of a red cell. They readily adhere to any surface, agglutinate in masses or rupture. Their sticky, agglutinative and fragile properties make the estimation of their number per cubic millimeter of blood extremely difficult and prone to inaccuracies. The most accurate techniques call for a quickly made blood smear which is stained and the proportion of the platelets and red cells counted. Then by doing a red cell count one can estimate the

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[1] Jour. Am. Med. Assn., 1924, **83**, 673.

number of platelets per cubic millimeter. This is a prolonged procedure and as no serious disturbance results until they diminish to one-half their normal number, less accurate methods suffice for practical information.

Principle of the Test.—The principle of the test utilized here is the same as the red cell count except that a special diluting fluid is used.

Apparatus:

Red cell pipet marked "101."
Spring lancet.
Alcohol, ether, and *vaseline.*
Diluting solution.[1]

This Must be Freshly Prepared Daily.—Keep on hand several papers containing 55 grains of sodium citrate. For occasional use this method is satisfactory.

For frequent use Rees and Ecker's fluid is best. It keeps indefinitely and is made as follows:

Sodium citrate 3.8 gm.
Formalin 0.2 cc.
Brilliant cresyl blue 0.1 gm.
Distilled water 100 cc. Filter before each count.

Outline of Technique.—Have the apparatus scrupulously clean. Fill the red cell pipet with citrate solution and immediately expel it to leave a film inside the tube and bulb. Draw in fresh blood to the 0.5 mark and citrate for dilution to the 101 mark. Shake and fill the counting chamber. After allowing ten minutes for the platelets to settle make a count of all the bodies one-fifth to one-half the size of a red cell under the high dry lens in 15 of the large squares which are each made up of 16 small ones. Divide the total counted by 3 and add 4 zeros to obtain the number of platelets in a cubic millimeter of blood. Normal 200,000 to 400,000.

Important Precautions.—It is essential to a good examination to prevent the blood from coming in contact with rough surfaces by using vaseline on the skin, to wipe away the first drop because many of the platelets are caught in the wound or ruptured by the tissue juices, and to fill the pipet as soon as the second drop of blood appears before the platelets are agglutinated by the air. Mix with the diluting fluid before many of them stick to the stem of the pipet.

Technique in Detail.—*Obtaining the Specimen.*—Have the pipets and counting chamber scrupulously clean. Clean the finger or ear lobe with alcohol and smear on a thin coat of vaseline. If the

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[1]

Sod. citrate	3 gm.	55 gr.
Distilled water	100 cc.	4 oz.

Filter, discarding the first few drops of the filtrate, which may contain small particles.

blood be drawn into the dry pipet many platelets will stick to the glass so it is best to moisten the inside of it by drawing in and expelling the citrate solution. The capacity of the pipet tube is not lessened. Even a red cell count is slightly higher by using a tube so moistened and the platelet count is higher by 100,000 per cu.mm. After this it is necessary to do all procedures as quickly as possible. Make a puncture that will bleed until it reaches exactly mark 0.5. Wipe away the first drop. Draw in the blood until it reaches exactly mark 0.5. Wipe the tip of the pipet. Plunge it into the diluting fluid, drawing up vigorously at first and later slowly to mark "101." Shake for a minute. After emptying the stem of the pipet put a drop of the contents of the bulb in the counting chamber and place it under the high power lens as directed in the count of the red cells.

The Count.—After allowing ten minutes for the platelets to settle adjust the light and focus on the red cells which appear greenish-yellow. Moving the diaphragm to admit slightly less light will bring the platelets into view. **The best aid in detecting them is to focus the fine adjustment slightly up and down to the red cells until the platelets appear as highly refractile glistening bodies, sometimes singly and sometimes in clumps** the latter being more difficult to count. Do not count anything smaller than one-fifth the diameter of a red cell. This is an arbitrary line between the platelets and blood dust or other granules. The top limit of a platelet diameter may be taken as one-half that of a red cell. As the platelets are few and irregularly distributed, it is best to count three times the area used for red cells, that is 15 large squares under the high dry lens, each made up of 16 small ones. Divide the total platelets counted by 3 and add 4 zeros to obtain the number per cubic millimeter. While the normal range is set down as 200,000 to 400,000 most of the normal counts will be 300,000 to 400,000 or over. If the examiner is not doing platelet counts frequently he should check his technique by seeing that a cubic millimeter of his own blood contains over 200,000 platelets.

Interpretation.—*Blood Platelets and Hemorrhage.*—Blood platelets play an important rôle in fibrin formation. If all the platelets could be removed from blood it would lose the property of spontaneous coagulation. When blood is placed under the dark-field microscope the platelets can be seen to burst like sky rockets and globules of protoplasm radiate in all directions. Fibrin threads appear along the course of these globules.[1] Unruptured platelets agglutinate when they touch a vascular defect and together with the other cells and fibrin aid in mechanically plugging the rent in the tissues.

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[1] Tait and Burke: Quart. Jour. Exp. Phys., 1926, **16**, 129.

Decreased Platelet Counts in Hemorrhage.—A decrease in blood platelets is found in essential or idiopathic purpura, sometimes known as essential thrombocytopenia or Werlhof's disease. It is thought to be due to an abnormal phagocytosis of platelets by the endothelial cells in the spleen rather than a deficiency in the parent cell the megakaryocyte in the bone-marrow. This cell has been found normal in fatal cases, while splenectomy produces a remarkable rise in the platelets and permanent freedom from symptoms. In this disease, which may be acute or chronic, the tendency to hemorrhage is the only symptom. There may be nose-bleeds, menorrhagia, gastro-intestinal hemorrhages or petechiæ. When the blood platelets fall to about 60,000 per cu.mm. there is a tendency to bleed but when they fall to around 10,000 the hemorrhages are severe. This is due to the lack of thromboplastin, a chemical contained in the platelets and a factor in clotting. There are remissions during which all symptoms disappear and the platelet count returns to normal.

In severe pernicious anemias or lymphatic leukemias there may be severe spontaneous hemorrhages associated with a low platelet count. This is due to the erythroblasts and lymphocytes crowding out of the bone-marrow, the megakaryocyte from which the platelets are formed.

Normal Platelet Counts in Hemorrhage.—Normal platelet counts may be associated with spontaneous hemorrhage and here the cause of the hemorrhage is usually a defect in the capillary wall. Serum sickness, scurvy, the symptomatic purpuras, peliosis rheumatica of Schonlein and the purpura of Henoch with its gastro-intestinal symptoms are examples of this state. In myelogenous leukemia and secondary anemias the platelet count is normal or increased. In infections, mild toxins stimulate the bone-marrow activity and increase the platelet output. Excessive toxins depress bone-marrow activity and so lower the platelet output.

In hemophilia the number of the platelets is normal but they are more resistant than those of normal blood. After hemophilic blood has stood in a paraffined tube for thirty minutes, smears of it show less of a decrease and less clumping of platelets than does normal blood.[1]

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[1] Howell: The Cause of Delayed Clotting in Normal Blood, Am. Jour. Physiol., 1926, **78**, 500.

Platelets and Immunity.—If an animal be given a large intravenous injection of bacteria which he can overcome and blood drawn from a vein in two minutes it will contain masses of organisms adhered together by many platelets. In eight minutes all organisms disappear from the blood stream. If the animal has no immunity to the bacteria injected, the platelets do not attack the bacteria which go on to multiply in the blood stream until the death of the animal ensues.

THE ESTIMATION OF THE RED CELL FRAGILITY.

Apparatus.—2 to 5 cc. hypodermic syringe with 22- or 23-gauge needle, both clean and dry.

Tourniquet, alcohol and absorbent, 1-cc. pipet.
4 ounces 1 per cent sodium chloride, 4 ounces distilled water.
12 small test tubes, about 3 inches by $\frac{3}{8}$ inch, in a rack.

The Test.—Prepare the various strengths of hypotonic saline as follows:

Tubes	1	2	3	4	5	6	7
c.c. of 1% saline	0.6 c.c.	0.7 c.c.	0.8 c.c.	0.9 c.c.	1.0 c.c.	1.1 c.c.	1.2 c.c.
c.c. of distilled water	1.4 c.c.	1.3 c.c.	1.2 c.c.	1.1 c.c.	1.0 c.c.	0.9 c.c.	0.8 c.c.
Resulting saline content in%	0.3	0.35	0.4	0.45	0.5	0.55	06
Normal degree of hemolysis	C.H.	C.H.	P.H.	S.H.			
Lessened hemolysis	C.H.	P.H.	S.H.				
Increased hemolysis	C.H.	C.H.	C.H.	C.H.	P.H.	S.H.	

C.H.= Complete hemolysis.
P.H.= Partial hemolysis.
S.H.= Slight hemolysis.

Insure a thorough mixing of the distilled water and 1 per cent saline by covering the mouth of the test-tube with the finger and inverting the tube twice. In each tube place a large drop of fresh unclotted blood and invert the tube to obtain a good dispersion of cells in the solution.

This blood may be obtained from a deep puncture incision on the finger-tip which will give 10 drops of blood. The more certain technique is to draw 1 cc. of blood from a vein with a thoroughly dry 22- or 23-gauge needle and hypodermic syringe and immediately place 1 drop from the syringe tip into each tube and shake. If the needle is not removed the drops will be smaller and 2 will be necessary. The results obtained with whole fresh blood are the same as those obtained with cells washed in saline.

Reading the Reaction.—After an hour or when the cells settle note (*a*) the dilution at which hemolysis begins as indicated by a very slight yellow coloring of the supernatant fluid just above the cells with a moderate deposit of cells at the bottom of the tube. Normal 0.4 per cent or 0.45 per cent. (*b*) The dilution at which hemolysis is complete as indicated by a deep red color of the supernatant fluid with an absence of any cell deposit at the bottom of the tube. Normal, 0.3 per cent. If tests are done infrequently a control test of cell fragility should be made on normal blood.

Interpretation.—In most cases of acquired and congenital familial hemolytic icterus with splenomegaly the resistance to saline of the red cells is below normal. On removal of the spleen these patients are relieved of their distress although their red cells still show a subnormal resistance to hypotonic saline. These patients on account of the marked hemolysis of their blood during crises frequently have gall stones. In pernicious anemia, splenic and other secondary anemias, especially the type due to gastro-intestinal cancer and in obstructive jaundice the resistance of the red cells to hypotonic saline solutions is above normal.

BLOOD CELL SEDIMENTATION TEST.

In blood containing an anticoagulant, the cells settle leaving a clear plasma. This sedimentation is more rapid in disease than in health. A measurement of its rate is of value in diagnosis and prognosis.

Apparatus.—1. Sedimentation tube—preferably the Cutler tube, which is 5 mm. in diameter, marked in millimeters beginning with zero at the 1-cc. level to 50 mm. at the bottom. Any tube of 2 mm. diameter or more will do. The larger the tube the more blood required. These tubes may be stood on plasticine or in a rack. If there are no millimeter markings on the tube a millimeter rule may be placed alongside to measure the cell drop.

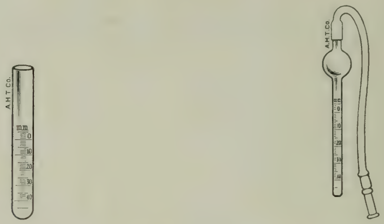
2. A 1- or 2-cc. hypodermic syringe graduated in $\frac{1}{10}$-cc. and a 23- to 24-gauge short needle.

3. Several ounces of 3 per cent sodium citrate.

4. Tourniquet, alcohol and gauze wipes.

5. Graph paper or Cutler charts.

Procedure.—Sterilize the hypodermic syringe and the citrate solution. Draw exactly 0.1 cc. of the 3 per cent sodium citrate into the syringe and fill up to the 1-cc. mark with blood from a prominent vein in the elbow. Draw in a large bubble of air and tilt the syringe to mix the citrate and the blood. If clotting occurs a fresh sample must be obtained. Pour the blood into the sedimentation tube, cork it and if there are more than one, label or number it. The blood will retain its usual sedimentation qualities for six to twelve hours, after which there is a slowing up of the rate.



Figs. 20 and 21.—Blood sedimentation tubes. Cutler models. Fig. 20, for venous blood, 1-cc. capacity; Fig. 21, for finger-tip blood, 0.3-cc. capacity.

Just before taking readings invert the tube several times to mix up the plasma and cells thoroughly and record on the chart every ten minutes for the first half hour the cell drop as indicated by the height of clear supernatant plasma. Another reading may be taken at the end of one hour.

Normal—men, 2 to 10 mm. and women 2 to 15 mm. in one hour. Changes occurring in the first thirty minutes are of the greatest importance.

Different Technique.—To obtain consistent results use for each test the same amounts of citrate and blood in a uniform shaped tube.

Variation in the sedimentation rate due to anticoagulants—

Heparin is the ideal anticoagulant. Hemophilic blood which will not clot for several hours will sediment at the same rate with or without heparin. Although only 1 mgm. of heparin is necessary to prevent coagulation of 5 cc. of blood, addition of 5 mgm. does not change the sedimentation rate. Its scarcity and high cost render it impractical for routine work.

Inorganic salts as anticoagulants retard the sedimentation in proportion to the amounts added. Sodium citrate in the amount advised above will give about 2 mm. less sedimentation in an hour. This will vary slightly with different samples of blood. Larger amounts of sodium citrate, potassium oxalate or sodium fluoride will retard the fall of cells to a degree to make the test very inaccurate. These salts are electrolytes and serve to suspend the cells in the plasma. The settling time is diminished even when blood is diluted with 0.9 per cent sodium chloride which has a density less than blood plasma.

Dilution of blood with plasma greatly increases the sedimentation rate by giving a more open path through which cells may fall, so that individuals with low blood cell counts and cell volume percentage have a rapid settling time. Other factors being equal, the cell settling time is roughly inversely proportional to the cell volume percentage.

The cause of the increased sedimentation rate in disease is still problematical. Excess of fibrinogen, albumin-globulin ratio, blood cholesterol, absorption of toxic products have all been considered.

Variation in Tubes.—Cells settle more rapidly in a tall column of blood than they do in a short one. The cells in a column of blood 200 mm. high (Westergren's technique) will usually settle 10 mm. while the same blood in a column only 50 mm. high (Linzenmeier) will settle 4 mm. The percentage of cell fall in each whole column is usually similar during a given time.

The diameter of the tube matters little. In tubes of 2.5 mm. diameter the boundary zone between the cells and plasma may not be as clear cut as in tubes of larger diameter and occasionally the cells settle at a slightly more rapid rate. The difference is negligible.

The narrow tube allows a micro technique using finger-tip blood. This may be useful if a vein cannot be located or the patient objects to venipuncture. To carry out this technique fill with 3 per cent sodium citrate and empty a collection tube of 1-cc. capacity. The amount clinging to the side will prevent coagulation. Make a small incision about $\frac{1}{8}$ inch deep on the postero-lateral side of the distal phalanx of the finger, preferably by means of an automatic lancet. Apply a tourniquet to the finger and collect about 0.3 cc. of blood. Agitate to prevent coagulation and cork. After shaking the vial well draw the blood up into a 1 cc. pipet or other narrow tube to make a column 50 mm. high. Stopper the lower end of the pipet with a cork, rubber band, gum or plasticine. Place the pipet in a vertical position and chart the sedimentation rate as with the larger tube.

Ordinary variations in room temperature produce only insignificant changes in the sedimentation rate. Aëration of the blood produces no change. Standing for six to eight hours at room temperature does not change the sedimentation rate but longer standing causes some retardation. Cells that have settled by gravity or even after centrifuging will, after a thorough mixing of the blood, settle at the same rate as they did formerly. The settling will occur at the same rate any number of times.

Recording Results.—Unfortunately there are at least three different methods of recording results. Fahraeus and Westergren recorded the **distance** through which the cells fell in one hour, usually 1 to 10 mm. If the fall be 10 mm. in a Westergren tube which holds a column of blood 200 mm. the **sedimentation index** would be 5 per cent.

Linzenmeier recorded the **time** for cells to settle a given distance of 18 mm. in a column of blood 50 mm. high. The normal is about two hours.

Cutler and others use a **graph** which is best because it shows any change in velocity, **especially the quick drops in the first twenty minutes which is so indicative of disease.**

Interpretation.—In one hour the normal sedimentation rate for men is 2 to 10 mm. with an average of 4 mm. For healthy women it is 2 to 15 mm. with an average of 6 mm. The cell sedimentation rate must be distinguished from the sedimentation time. When the rate is increased, the time is lessened.

The sedimentation rate gives somewhat the same information as a leucocyte count, except that, if the disease continues for weeks, the rate will remain high while the leucocyte count falls to normal.

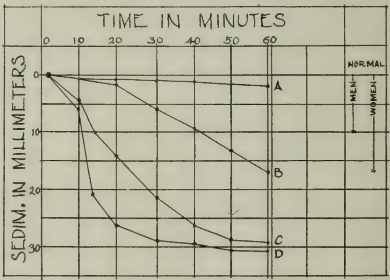


FIG. 22.—*A*, horizontal line, clinically healthy individual; *B*, diagonal line, clinically quiescent infection; *C*, diagonal curve, clinically slightly active infection; *D*, vertical curve, clinically markedly active infection.

The rate is increased in acute inflammation, active chronic inflammation such as tuberculosis, malignancy and in the last four months of pregnancy. The increase in settling appears mostly in the first twenty to thirty minutes, so that the graph is almost L-shaped in contrast to the normal, which is a gradual slope.

An increased sedimentation rate indicates destruction of body tissues but it does not diagnose which specific disease is causing this increased rate, any more than a thermometer will tell what special disease is the cause of a fever.

In the *acute inflammatory conditions*, the rate of sedimentation is usually directly proportional to the extent and severity of the inflammation. It may settle 24 mm. in fifteen minutes in severe cases. If the inflammation is extending the sedimentation time decreases. A slow rate eliminates infection as a cause of the symptoms. In its early stages appendicitis does not give as rapid a cell settling as salpingitis or pelvic cellulitis. The likely cause is that appendicitis is localized to a small area at first, but when it involves the surrounding structures the settling time is always decreased. Readings for one week after a surgical operation show an increased rate due to the tissue trauma and absorption. If the rate continues high after the first week it indicates an infectious complication.

In *gynecology* it may aid in determining the best time to do non-urgent operations. A patient showing a sedimentation of over 18 mm. in an hour, if operated on for pelvic inflammatory disease, may have a stormy convalescence. A reading of 18 mm. in thirty minutes is usually found in the presence of acute pelvic abscess, and if the case is operated on at all, conservative evacuation should be employed. In the differentiation between ectopic pregnancy and acute exacerbation of a chronic inflammatory process, a rapid sedimentation points toward the infection, while a slow sedimentation points toward ectopic. In uterine fibroids and ovarian cysts the sedimentation rate is normal provided there is no complicating degenerative changes or infection. With these complications the rate is increased. Readings are valueless after the third month of pregnancy and for the first two weeks of the puerperium, as a low reading cannot be attributed to an infection. Although the sedimentation time is increased after the fourth month of pregnancy the physical signs of that condition are a much more definite means of diagnosis.

The sedimentation rate is not a good means of differentiating between pregnancy and fibroids or ovarian cysts because the complications which may attend fibroids and cysts give an increased rate like that of a pregnancy. A normal rate is less fallacious in this differentiation.

The chief chronic inflammatory disease in which we use the sedimentation test is *tuberculosis*. The sedimentation rate parallels the degree of anatomic involvement of the lung. In estimating the prognosis, if the sedimentation rate be increasing the tuberculosis is progressing and extending. If the sedimentation rate be lessening and becoming more normal the tuberculosis is becoming quiescent. In a busy clinic the test may be of value if performed at monthly intervals to determine the course of the disease.

In *anemias* the sedimentation time is shorter than normal. The rapidity is directly proportional to the severity of the anemia, and this is probably sometimes a factor in producing the shorter sedimentation time in chronic disease.

In general, *malignant tumors* show an acceleration of sedimentation rate to over 18 mm. in one hour, while benign tumors do not. Early malignant processes do not show a decreased blood settling time, but later the settling time decreases as the malignant process extends, probably due to infection of the degenerating areas. Physical and *x*-ray examinations are better means of diagnosing a malignant process.

Although the sedimentation rate is a valuable index of disease, it should be regarded only as *one* important sign. Clinical symptoms and physical findings cannot be neglected. The rate from day to day sometimes varies in chronic disease out of all proportion to any change in the patient, in the same manner as the antibody content of the blood will show daily variations by the complement-fixation test.

In *jaundice* or *hepatic disease* the sedimentation rate is usually increased but in *cyanosis* or *polycythemia* from any cause, it is slower than normal.

For those who use the *time* method, about two hours should be considered a normal time for the erythrocytes to settle 18 mm. in a column of blood 50 mm. high. Dr. Polak[1] used this method of recording results, and emphasized the value of the test in gynecology by stating that it is more easily performed and gives more information than the leucocyte count in the presence of infection. He says an operation for infection should not be done when the reading is less than ninety minutes. In ectopic pregnancy the sedimentation time is usually over one hundred minutes. When the settling time is thirty minutes localized purulent material is always found. Around the hour limit there is usually a quiescent infection which may be activated by surgical operation. Even when it is below ninety minutes the morbidity is greater, complications more frequent, and convalescence prolonged. Patients should not be discharged from hospital if their sedimentation time is less than forty-five minutes.

BLOOD GROUPING.

PRINCIPLE.—All human bloods may be divided into four groups. The serums of some groups will agglutinate the cells of others. (See table page 83.)

Apparatus and Reagents.—Serums of groups A and B (2 and 3).[2]

One ounce of 0.9 per cent sodium chloride and 1 per cent sodium citrate in distilled water.

Two small test-tubes.

Two glass slides.

Blood lancet and microscope.

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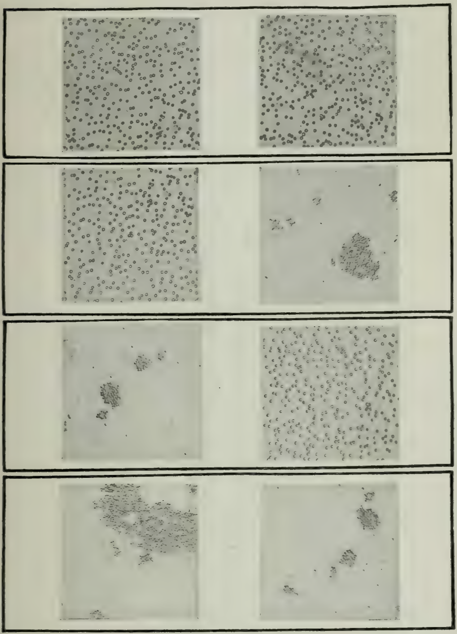
[1] Polak and Tallefson: Clinical Significance of the Sedimentation Test, Jour. Am. Med. Assn., 1928, **90**, 168.

[2] One cubic centimeter of each is sufficient for about 20 tests. These typing serums should have high agglutinating powers, and if stored in an ice-chest, in hermetically sealed capillary tubes or vials, will remain potent for one year. They may be obtained from hospital laboratories or biological houses. Lederle Laboratories market 10 capillary tubes each of A and B for $2.00.

To Determine Blood Groups. One drop of emulsion of cells (1 drop of blood in 2 cc. of 3 per cent sodium citrate) added to 1 drop of:

Serum from blood group A (2) (containing *b* agglutinin).	Serum from blood group B (3) (containing *a* agglutinin).



	Landsteiner	Moss	Jansky
Blood group	0	4	1
Blood group	A	2	2
Blood group	B	3	2
Blood group	AB	1	4

FIG. 23.—Appearance of red cells under the low-power lens of the microscope in five to fifteen minutes.

The Test.—To 2 cc. of the citrate saline solution in a small test-tube add 1 drop of fresh blood from the ear lobe or finger-tip. If the patient be anemic add 2 drops of blood instead of 1. Invert the tube several times to mix the cells. Mark one end of a glass slide A and the other end B. At the A end, with a clean pipet, place a drop of Group A (2) serum and at the B end a drop of Group B (3) serum. Into each serum by means of a small pipet place a drop of the cell emulsion. Tilt and gently rotate the slide so that the cells and serum are well mixed. Repeat this in two minutes. Agglutination, if it occurs shows up plainly in from five to fifteen minutes. Where it is marked, a fine brick dust is often visible to the naked eye. Place a coverslip over the mixture and under the low power of the microscope, using subdued light, examine to confirm the naked eye appearance.

Interpretation.—*If neither serum agglutinates the cells the blood belongs to Group C (Group 4, Moss) which is a universal donor.* If only the serum of B shows agglutination the cells belong to Group A (2) but if the cells are agglutinated only by A they belong to Group B (3). When both serums agglutinate the cells they belong to Group AB (Group 1, Moss).

Important Facts.—*Permanency of Groups.*—The blood groups are remarkably permanent throughout life and are not influenced by drugs, surgical operations or protein injections. If a patient has received a transfusion his blood may have the group characteristics of the blood which he received as well as his own.

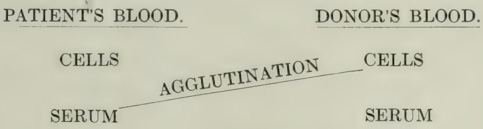
Blood Groups in Infants.—Agglutinogen, the agglutinable factor in red cells is usually present at birth, Agglutinin, the agglutinating factor in the serum usually develops after birth. Occasionally however, it is present at birth. It is the rule for it to appear in about eight days but in some infants it does not appear until two years of age. If this agglutinin be present in the serum of an infant it will agglutinate the cells of any donor who is not either in the O group or in the same group as the infant. Such an agglutination if marked will cause death after transfusion.

Practical Application.—The chief value of blood grouping is to eliminate unsuitable donors. Make a survey of the young men in the community for probable donors. They should be twenty to thirty years of age, free from every kind of disease and have prominent veins in the forearm. Do not wait until a transfusion is needed but group them at the earliest opportunity. By keeping a record of the groups one can, in an emergency transfusion, make use of a universal donor at a moment's notice without further testing.

Never transfuse blood without knowing the group of the donor. The only possible exception to this is in continuing hemorrhage of the new-born where the subcutaneous injection of blood has failed and the equipment for blood grouping or blood matching is not at hand. Because most infants have no agglutinin in their blood at birth the danger from transfusion is less than the danger from the severe hemorrhage.

In an emergent hemorrhage in adults one may use the universal donor but it is not so satisfactory as when both donor and patient are in the same group. If a universal donor be used the patient may have a chill and fever following the transfusion. When time and facilities permit it is best to do a direct blood matching as an extra check on the blood grouping. In this way the effect of the patient's serum on the donor's cells and the donor's serum on the patient's cells may be observed. A partial agglutination occasionally occurs between the bloods of individuals in the same group due to the presence of sub-groups in the A (2) and B (3) groups, so that a direct matching should be done before each and every transfusion.

THE DANGER IN TRANSFUSION.



BLOOD MATCHING.

PRINCIPLE OF BLOOD MATCHING.—One individual's serum may agglutinate another's cells. The phenomenon, if present, may be seen by viewing a drop of the serum and cell emulsion under the microscope, that is, the patient's serum and the donor's cells; also the patient's cells and the donor's serum. The effect of the patient's serum on the donor's cells is the more important.

DIRECT MATCHING OF BLOOD FOR AGGLUTINATION.

SIMPLE METHOD.[1]—PRINCIPLE.—The cells of the recipient's blood are hemolized by chloroform which soon evaporates. Then a very small drop of the donor's cells is placed in the recipient's hemolized blood and, after mixing, it is examined under the microscope or with a hand lens for agglutination of the cells.

Apparatus.—A blood lancet, glass slides, chloroform, a hand lens or microscope.

Technique.—Clean the finger and after it dries puncture the dorsum between the nail and first joint. Place a large drop of blood on a glass slide, cover it with several drops of chloroform and mix with a wooden applicator or match end. This insures the hemolysis of the recipient's red cells.

When the chloroform evaporates place a small drop, pin-head size, of the donor's blood on the drop of recipient's hemolized blood and tilt the slide to mix. If agglutination occurs it does so in less than five minutes. Examine for the clumping of the cells under a hand lens or the low power of the microscope. Repeat the test with the hemolized blood of the donor and the cells of the recipient.

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[1] Clements, J.: Zentralbl. f. Chir., 1926, **53**, 3032.

Precautions.—The drop of unhemolized blood to be tested for agglutination should be very small. If it is large, the cells are so close that it is impossible to see agglutination. The intense red color of the hemolized serum is much like the cells and by adding 2 drops of 0.9 per cent sodium chloride or 3 per cent sodium citrate to the hemolized blood after the chloroform a clearer picture is given of any agglutination taking place in the added cells. The examiner should be convinced in his own mind that he can recognize agglutination by this method before he uses it to determine the suitability of a donor.

LABORATORY METHOD. *Apparatus.*

Lancet.
2 ounces 0.9 per cent saline.
2 small test tubes.
2 vials or glass capsules to hold 4 to 8 drops.
Centrifuge, glass slides, hypodermic syringe and needle.
Microscope.

The Test.—Place 2 cc. of saline in a test tube. Make a puncture incision into the ear or finger of the patient and place 1 drop of blood into the 2 cc. of saline and 4 to 5 drops of blood in the capsule or vial. Label both P. for patient. Obtain the blood in the saline and in the capsule from the donor in a like manner. After the blood in the capsule or vials has clotted, centrifuge, and with the aid of a hypodermic syringe or capillary pipet mix on a glass slide 1 large drop of patient's serum with one of donor's cells. Mark it P.S. and D.C. At the other end of the slide mix a drop of donor's serum and patient's cells. Tilt the slide with a rotary motion and examine under the low power of the microscope. Clumping occurs in five to fifteen minutes. (See page 83.) Rarely hemolysis takes place and no red cells may be seen at all.

Interpretation.—Either clumping or hemolysis of the donor's cells by the patient's serum renders a transfusion so dangerous that it is liable to be fatal. Hemolysis allows the sudden release of an altered protein into the blood stream. Masses of clumped cells act like multiple emboli. These produce a state of sudden shock and frequently death. Clumping of the patient's cells by the donor's serum does not mean that a transfusion is dangerous but it will be attended with a fever and considerable reaction. In transfusion the donor's serum is so diluted by the patient's blood and "fixed" by his body cells that it does not exert enough agglutinating power on the patient's red cells to be dangerous. No clumping of the donor's serum gives the greatest assurance of safety and freedom from reaction.

BLOOD TRANSFUSION.

Indications.—1. Sudden loss of a considerable quantity of blood. Transfusion is the most successful curative measure for this state.

2. Surgical shock. Here it is necessary to give the transfusion within a few hours of the onset of the shock before the vital centers become damaged.

3. Anemias of slow onset. Here transfusion may be given with benefit if the hemoglobin be below 60 per cent. It is especially valuable as a pre-operative measure. It improves the resistance of the patient. In pernicious anemia transfusion is a palliative measure only and in the leukemias transfusion is of doubtful value.

4. Hemorrhagic disease of the new-born is promptly arrested by a transfusion of blood. Hemorrhagic diseases of adults are controlled, the severe forms for about a week, the mild forms for several weeks.

5. Transfusion is a valuable remedy for carbon monoxide or coal-gas poisoning. Small repeated transfusions of 100 cc. each are sometimes helpful in acute septic infections. Its value is doubtful for hemorrhages from enteric fever, in eclampsia or uremia.

Transfused cells may live for four months in the patient if they match perfectly. If not they disappear more readily. The main functions transfusion performs are increased oxygen-carrying power, increase in the bulk of the circulating fluid and the stimulus to blood formation.

The Choice of a Donor.—The donor should not have any fever and syphilis should be ruled out by a physical examination and a blood Wassermann test done immediately before the transfusion. If the transfusions are to be repeated it is better to use a different donor each time because the patient may become hypersensitive to the blood of one individual especially if several weeks elapse between the transfusions. The donor should be in the same group as the recipient as shown by grouping and direct matching tests but in cases of great emergency or if no donor be available of the same group as the patient a universal donor of Group O (4, Moss) may be used. Donors suffer no ill effects from giving several 500 cc. transfusions at monthly intervals.

Caution Regarding Transfusion.—Except in cases of emergency, as well as cell grouping direct matching of the blood of donor and recipient should be done to eliminate reactions from sub-groups and high agglutinating power of the donor's serum on the patient's cells. Blood from a donor of an incompatible group will cause a severe reaction in the patient often one minute after 5 to 10 cc. are introduced. The earliest symptom is a slight cough followed by a sense of weight and constriction in the chest. This can be caused by too rapid introduction of the blood. There may be abdominal and lumbar pains, deep and labored breathing and disturbances of the pulse-rate. Blood should be administered slowly at first and if the patient coughs two or three times or feels any uneasiness the transfusion should be stopped. There are sometimes reacting substances in the blood which the usual agglutinating test does not demonstrate. The best remedy for any untoward symptoms is 0.5 cc. (7 minims) of adrenalin injected hypodermically.

Minor reactions occur occasionally and are characterized by a chill and 2 or 3 degrees of fever. They are due to citrate reactions or the action of the donor's serum on the patient's leucocytes or *vice versa.* The technique of this test is too extensive to warrant use.

It is best that the donor should have a fasting stomach at the time of transfusion, as the turbidity occurring in the plasma after a meal may give a reaction.

Technique of Transfusion.—The Direct Method.—The direct method at its simplest requires two operators; one to take the donor's blood and the other to inject it into the patient.

Apparatus.—Four 20-cc. glass syringes with two 18-gauge and two 20-gauge needles.

Vaseline.

One pint 0.9 per cent sterile saline, local anesthetic and hypodermic syringe, 2 tourniquets, 2 sets of rubber gloves, 2 towels, all sterile.

Procedure.—Mark the hub of the needles to indicate on which side the point is bevelled. Put on rubber gloves and draw hot sterile vaseline into the lumen of the syringe and needle. Expel any excess. Place the syringes and needles on a sterile towel. Have a tourniquet applied to the arms and after washing the antecubital fossa with alcohol inject a small drop of local anesthetic between the layers of the skin just alongside of the most prominent vein. Through this wheal insert the large needle into the vein and when the blood runs, cover the end of it with the thumb. One operator fills the syringes with the donor's blood, passes it to the other who fits it to the needle already in the patient's vein and injects the 20 cc. of blood in half to one minute. The needles should be held as steadily as possible so they will not move out of the lumen of the vein or injure its wall. If the bevelled side of the needle point lie flat against a vein wall one cannot withdraw blood. By noting the mark on the needle hub one can prevent this. When the syringe is full separate it carefully from the needle and place the thumb over the needle outlet to prevent loss of blood while the full syringe is being passed to the operator injecting blood into the patient and an empty syringe is taken up.

When it is impossible to introduce the needle by the above procedure the following will succeed: Apply the tourniquet tightly above the elbow. Then wind an Esmarch's tourniquet or gauze bandage around the wrist and with a gradual spiral up the arm. This distends the veins at the elbow even when the patient is in a state of collapse and a needle can easily enter the vein.

The Citrate Method.—This method is simpler and preferable as it requires only one operator. After instilling a drop of local anesthetic between the layers of the skin a large needle, 15- to 18-gauge, should be inserted into the donor's vein and the blood allowed to flow into a glass vessel containing 50 cc. of 2.5 per cent solution of sodium citrate. This will prevent coagulation of up to 450 cc. of blood provided it be well mixed by stirring as the blood falls into the citrate solution. Strain the citrated blood through 2 to 4 layers of sterile gauze to remove any clots that may have formed and inject it into the patient by means of a glass funnel connected by a rubber tube to a needle, 20- to 21-gauge, which is inserted into the patient's vein at the volar surface of the elbow. A rubber tube 18 to 24 inches in length will give sufficient gravity to cause the blood to flow into the vein. All apparatus may be sterilized by boiling several minutes in 0.9 per cent sodium chloride.

The usual amount of a transfusion is 500 cc. but from 300 to 800 cc. may be used. Fifty to 150 cc. may be used for children.

In infants it is difficult to find a vein. Mild cases of hemorrhage of the new-born respond to from 20 to 30 cc. of blood injected subcutaneously, for which the bloods do not require matching. In the more severe cases massive transfusion is necessary and the bloods have to be matched. The veins in the forearm are too small and the superior longitudinal sinus as a route is dangerous. There are numerous cases on record where the blood was injected into the skull cavity instead of into the sinus. The external jugular vein can be used. If the baby cries while lying on his back with his head turned to one side the external jugular vein will stand out. A tourniquet lightly applied low down on the neck will aid this. It is just under the thin platysma muscle and runs from the mandible to the middle of the clavicle. If one cannot gain entrance to this vessel the peritoneal cavity does not clot or form adhesions and the cells are absorbed into the circulation in a comparatively short time without any change in their structure, if the bloods be compatible. The site of injection is $\frac{1}{2}$ inch below the umbilicus, slightly lateral to the midline.

The amount of the transfusions and their frequency should be governed by hemoglobin estimations or red cell counts.

Tissue Grafting.—Tissue grafts grow more readily when taken from an individual who has the same blood group as the patient. Autogenous tissues are the most successful.

Medico-legal Use for the Grouping of Blood Cells.—It is often possible to determine the blood group of blood stains on garments or floors for comparison with the blood group of a suspected criminal.

Technique.—Wash the cells with normal saline and if they appear distinct under the microscope try the effect of Group A and of Group B serum upon them. An hour is necessary because of the possible dilution and it is best to use a hanging drop to prevent evaporation. When the cells are disintegrated so that their agglutination cannot be detected immerse the particles of blood stain in a minimum of saline, filter and try the agglutinative effect of the filtrate on cells known to be in A and B groups.

If the cells of A and B groups are agglutinated the unknown blood is Group O.

If the cells of A groups are agglutinated the unknown blood is Group B.

If the cells of B groups are agglutinated the unknown blood is Group A.

If the cells of neither groups are agglutinated the unknown blood is Groups AB.

No value can be attached to non-agglutination because this may be due to too great a dilution of the blood stain.

Cases of Disputed Parentage.—An agglutinogen (AB, A or B) never appears in a child unless it be present in one of the parents. If the blood group of one parent and the child be known, in certain cases the group of the other parent can be determined.

If the blood group of parents be	then	Blood groups of children must be	and	Cannot be
AB and AB		AB, A, B or O		
AB and A				
AB and B				
AB and O				
A and B				
A and A		A or O		AB or B.
A and O				
B and B		B or O		AB or A.
B and O				
O and O		O		AB, A or B.

This method is on a sound foundation but is not, as yet, much used in medico-legal work.

Theoretical Considerations of Blood Groups.—The agglutination which occurs in blood grouping and matching is sometimes referred to as isohemagglutination. The "iso" limits the reaction to one species of animal, in this instance the human. The "hem" specifies that the agglutination is of blood cells.

In 1907 Jansky made a classification of the blood groups numbering them 1, 2, 3, and 4. As his work was not in English and escaped recognition, Moss, independently placed bloods into four groups in which Groups 1 and 4 of Jansky were reversed. This has caused great confusion especially regarding universal donors which are

Group 1 Jansky and Group 4 Moss, so that it is perhaps better to follow Landsteiner's grouping by the iso-agglutinogen in the red cells as O, A, B and AB. The important factors are A and B, the agglutinable substances in the cells of the different groups. As the result of a questionnaire to many hospitals Kennedy[1] recommends that the Moss system, where Group 4 is universal donor, be universally adopted to avoid confusion.

Percentage of individuals.	Equivalent blood groups.			Agglutinogen in red cells.	Agglutinin in serum.	May receive from	May give to
	Landsteiner.	Moss.	Jansky.				
45	O	4	1	O	ab	O	All
40	A	2	2	A	b	O and A	A and AB
10	B	3	3	B	a	O and B	B and AB
5	AB	1	4	AB	o	All	AB

Cells containing A agglutinogen are agglutinated by serum containing (a) agglutinin but not by serum containing (b) agglutinin.

Occasionally individuals both in Group A may show a reaction when their bloods are matched. This is due to an additional sub-agglutinogen and sub-agglutinin, sometimes named C. Such reactions are not strong and their presence has frequently been disputed. The evidence in the favor of sub-groups make direct matching prior to transfusion desirable in order to prevent minor reactions. The serum of one person may hemolyze the leucocytes of another. This may cause a chill and fever but is not dangerous. The test for leucocytic reaction is too tedious for routine use.

Cases have been reported where an individual's serum has agglutinated his own cells at room temperature. Recently Japanese workers[2] have reported this phenomenon as a cause of Raynaud's disease. I have tested the blood of 2 such cases but no agglutination occurred at room or ice-box temperature.

THE ANEMIAS.

(Diseases of the Hemic System).

Anemia in the early stages is a reduction in the blood coloring matter, the hemoglobin. In the advanced stages both the hemoglobin and the red cells are diminished in a given volume.

[1] Jour. Am. Med. Assn., 1929, **92**, 610.

[2] Iwai and Meisai: Japanese Med. World, 1926, **6**, 345.

This deficit may be due to loss from hemorrhage, an excessive blood destruction in the spleen and liver or a lack of proper blood formation in the bone-marrow. The bone-marrow which produces the red cells is of vital importance in blood disorders and the term blood diseases is being replaced by diseases of the hematopoietic system. This is with the idea of assigning the disease to its proper site in the same way as we refer to bronchiectasis rather than purulent sputum and nephritis rather than albuminuria. As the most frequent blood disorder is due to hemorrhage and as excessive hemolysis of the formed blood cells is an accepted phenomenon, it is more correct to use the wider term of hemic system, rather than to use the term hematopoietic which refers only to the origin of the cells.

Anemia as a term will probably remain because the blood deficit is an outstanding feature of the disorder and blood examination more practical than the examination of bone-marrow sections.

Appearance in Anemia.—Only in marked anemia is the appearance of a patient a guide to the blood state. Not all pale people are anemic, neither are all anemic people pale. The color of the skin partially depends upon its own pigment and the depth of the bloodvessels. Frequently in fat people the bloodvessels are deep and the skin appears pale. The color of the mucous membranes, of the lip and of the palpebral conjunctiva, while more reliable indicators than the skin, also appear pale at times when the hemoglobin and red cells are normal. This is frequently found in the state of fear or anxiety or in any diseases where there are contracted peripheral arterioles.

When the patient is lying down, the lip and conjunctiva, in the slightly anemic, show more color than when the patient is sitting or standing.

A false plethoric appearance is present in patients who have a slight degree of jaundice and it is sometimes present in those who have deeply pigmented skins.

If there is a slight irritation of the palpebral conjunctivæ they appear more florid than the blood state by itself would indicate. The appearance of the palm is the most reliable clinical guide and is given in detail below. Even after observation of the palm color however, one should do an actual blood examination before making a definite diagnosis of anemia.

THE PALM COLOR TEST. (DUKE.)—PRINCIPLE.—The palm of a patient held at the level of his heart is compared with a NORMAL palm similarly held. Anemia and plethora cause variation of color from the normal. The palm color does not vary with age, sex, complexion or occupation.

Standard.—The physician's hand is used as a standard and he must make sure that his palm color is normal by blood examination or comparison with the palms of healthy young adults or when possible by both methods. The patient's hand should be semiflexed with the palm upward and held about the level of his cardiac apex. This is compared with the physician's hand held in a similar manner at the level of his cardiac apex. It is essential that the hand should be relaxed to the semi-flexed position. Forced opening of the hand produces a general pallor of the palm as well as many white spots. Lowering the hand increases the plethora of the palm. When the hands are in position at the level of the cardiac apex, allow ten to twenty seconds for the circulation to stabilize before making the estimation.

Interpretation.—A pale palm indicates secondary anemia and a deep red palm indicates plethora or polycythemia. This is more accurate than observation of lips, conjunctivæ or skin because the circulation to the palm held at a given level is constant and the physician's normal palm acts as a standard for comparison whereas in judging the pallor of other membranes the variations due to pigmentation, subcutaneous fat, vasodilatation and constriction interfere and the physician's mental impression becomes indefinite without a standard for immediate comparison. The palm color test is an estimation of the hemoglobin intensity or percentage in a bed of capillaries where variation is at a minimum.

With even mild degrees of secondary anemia where the hemoglobin is 60 per cent and there is no change in the red blood count the palm will be definitely more pale than the normal and when the red cell count is much below 4 million the patient's palm is almost paper white. There are some states in which the palm color test fails us. In Addison's pernicious anemia where the blood counts are frequently 2 or 3 million, the palm color may be normal. The probable reason is that a count of the cells from the finger-tip is frequently 50 per cent higher than a red cell count of venous blood in the same patient. This difference is due to the tendency of the macrocytes to lag in the capillaries.[1] A drop of blood obtained from the capillaries after a pin-prick gives much the same cell count as blood obtained from the vein in most other states of health and disease except in edema, when the capillary count is lower due to the admixture of lymph from the tissues. In jaundice or cyanosis the palm color test will not indicate any anemia that may be present. The palm color is normally slightly higher than average in the Latin races and in obese patients past middle age.

The test may be used to determine the amount of blood that should be given in a transfusion.[2]

Blood Examination Does Not Give Full Information.—Overinterpretation from examination of a small amount of blood is at present an all too common fault. It will lead to frequent errors in diagnosis and prognosis. Such reports are at times accepted as from an oracle while the story of the illness and the whole disease-ridden body is not carefully investigated. Each result of a laboratory examination is only one special physical sign. Some are of great importance, others of slight importance.

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[1] Duke and Stofer: Arch. Int. Med., 1922, **30**, 94.

[2] Duke: Palm Color Test, Arch. Int. Med., 1928, **42**, 533.

Healthy bone-marrow may keep blood counts up to normal even when there is considerable hemorrhage or hemolysis. This state is known as a compensated anemia and it is attended by an increase in the reticulated red cells. Where the hemorrhage is slight but the anemia marked there is usually another cause such as poor nutrition or a toxic infection. If there is a hemolytic toxin present it may inhibit the formation of the red cells as well as having a hemolyzing effect on those in the blood stream. Idiopathic pernicious anemia has been regarded as a definite hemolytic process but recent information derived from bone-marrow studies and results of liver diet indicate that the defect is in the bone-marrow itself. Hypoplastic anemia, where there is poor regeneration of red or white cells, points to a deficit in the blood-forming tissues. When ulceration of the throat due to Vincent's organisms is associated with aplastic anemia it is frequently difficult to tell which is primary, the anemia or the infection.

DETERMINING THE CAUSE OF ANEMIA.—Sometimes the cause of an anemia is very apparent. There may be a gross hemorrhage, obvious disease, the ingestion of a poison or faulty diet and hygiene. Physical examination may reveal a cancer of the rectum, a mass at the pylorus of the stomach, a palpable spleen or enlarged lymph glands.

All too frequently the cause is obscure and anemia is the only sign present. One or MORE of the following may be a cause, slight intermittent hemorrhage, low-grade focal infection, obscure new growth, ingestion of a poison or faulty nutrition. For this an extensive systematic examination, as shown in the following table, is necessary.

Inquire and examine carefully for:

HEMORRHAGE.

Gastro-intestinal: History of blood vomited or passed by bowel. Gross examination of gastric contents and stool for blood. Chemical test of stool for occult blood. X-ray examination; after opaque meal or enema.

Uterine.—History of menorrhagia or metrorrhagia.
Examination for fibroids or other gross causes.

Hematuria.—Urinary examination.

Epistaxis.

TOXIC PROCESSES.

External.—Occupation; unhygienic surroundings—lead contact; benzol in manufacturing or dry cleaning; mercury or arsenical drugs.

Internal.—Infection; temperature, pain, pus discharges or inflammation.
Focal infection, endocarditis or syphilis.
Parasitic infections. Stool examination.
Nephritis; urinary or cardiovascular examination.
Thyroid disturbances; goiter or myxedema; basal metabolism.
New growth; with ulceration; interfering with nutrition or involving bone-marrow. Physical and x-ray examination.
Splenic tumor.

Nutrition.—Write down the articles of diet and the amount taken by the patient on the day of the examination and on the previous day. He can state this accurately. Inquire whether it is his usual routine. Note any deficiency of protein, fat or carbohydrate and whether sufficient vitamin is included.

Liver diet as a test; in pernicious anemia the patient improves in one week; in other anemias, more slowly.

CLASSIFICATION OF ANEMIAS.

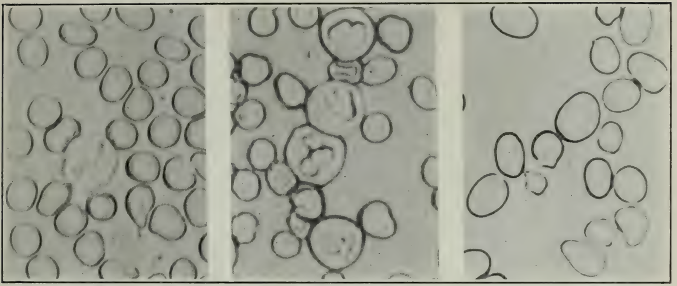
As the blood does not respond to each special irritant in a special manner, identical blood pictures occur from widely different causes. The intensity of a single irritant may vary, producing widely different blood pictures. Small amounts produce stimulation; large amounts, destruction. Any classification on hematological grounds will therefore be faulty. There are few pathognomonic blood pictures but there are many containing important information.

- **Hemorrhagic Anemia.**
  - **Acute. (Dealt with on page 183.)**
  - **Chronic.**
- **Hemolytic Anemia.**
  - **Acute infections.**
  - **Malaria. (Dealt with on page 44.)**
- **Anemia Due to Defective Cell Formation and Hemolysis.**
  - **Hemolytic jaundice.**
  - **Addison's anemia.**
  - **Anemia of pregnancy.**
  - **Tapeworm anemia.**
- **Anemia Due to Defective Cell Formation.**
  - **Anemia of malnutrition,**
  - **Chlorotic anemia. (Iron deficit.)**
  - **Toxic anemia from chronic infections, malignancy, nephritis, myxedema and lead poisoning.**
  - **Hypo- or aplastic anemia, anemia from severe hemorrhage or sepsis, benzol, x-ray or arsenical poisoning.**
  - **Myelophthisic anemia.**
  - **Leukemic anemia.**
  - **Sickle cell anemia.**
- **Anemia of Childhood.**

Chronic Hemorrhagic Anemia.—These patients complain that they are weak and very easily fatigued. They may also complain of palpitation, breathlessness, dizziness, edema of the face or extremities. There may or may *not* be a history of bleeding. Pallor is present when the anemia is moderately advanced. Small repeated hemorrhages from the gastro-intestinal or genito-urinary tract produce an anemia more readily than bleeding into serous cavity or from the lungs. If there is much bleeding over a long period of time the cell reproductive power of the bone-marrow may become exhausted.

In experimental work it is remarkable how long a well-fed animal will bear repeated loss of small amounts of blood without a resulting

Unstained and unmounted. Diminished light.

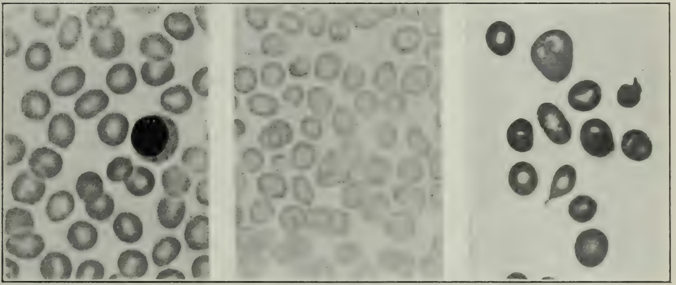


Normal

Leucocytosis (25,000)

Anemia, (slight anisocytosis)

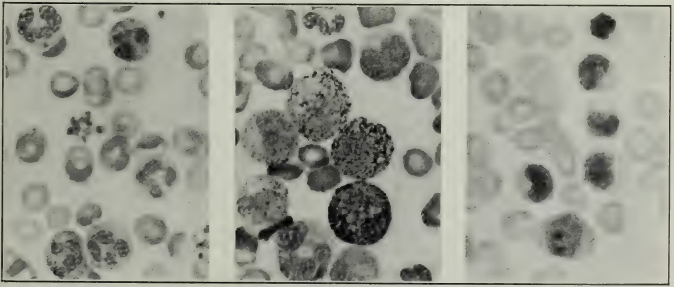
Stained by Wright's and Mounted in Balsam.



Normal

Hypochromic anemia

Hyperchromic anemia (Addison's)



Leucocytosis

Myelogenous leukemia

Lymphatic leukemia.

FIG. 24.—Blood cell pictures × 500 1 mm. = 2 microns

anemia. If the diet be scanty, ill-balanced or poor in iron the anemia readily develops. In anemia associated with hemorrhage the diet is a more important factor than we have formerly considered it. Well-nourished bone-marrow can compensate a moderate drain on the blood.

Blood Findings.—The most outstanding feature of the blood smear is very pale cells; many having only a narrow ring of cytoplasm at the margin. There are moderate changes in the form and size of the cells with many small forms. Special staining will show 3 to 5 per cent reticulocytes if the bone-marrow is not worn out. The hemoglobin is more reduced than the red cells making a color index under 1. The blood platelets are very numerous whereas in pernicious anemia they are scanty. This difference is so marked that it can be detected on the unstained smear. The leucocyte count is about normal with at times a tendency to lymphocytosis indicating an overworked state of the bone-marrow.

TYPICAL BLOOD PICTURE OF CHRONIC ANEMIA FROM HEMORRHAGE.

	Slight.	Severe.
Hemoglobin, per cent	**65**	**25**
Red blood cells:		
Million per cubic millimeter	3.8	2.5
Color index	**0.8**	**0.5**
Blood spread:		
Achromia	*Moderate*	*Marked*
Poikilocytosis	None	Moderate
Anisocytosis	*None*	*Slight*
Microcytes	None	Present
Macrocytes	None	Few
Normoblasts	None	Present
Megaloblasts	None	None
Polychromasia	None	None
Stippling	None	None
Reticulocytes, per cent	2	5
Icterus index	4	2
White blood cells:		
Thousand per cubic millimeter	6	4
Differential count:		
Lymphocytes, per cent	20	30
Monocytes, per cent	5	5
Eosinophile polymorphonuclears, per cent	3	1
Neutrophile polymorphonuclears, per cent	72	62
Segmented, mature	65	50
Band nucleus, young	7	12
Myelocytes, per cent	0	2
Undetermined, per cent	0	2
Platelet, thousand per cubic millimeter	300	250

The diagnosis of chronic hemorrhagic anemia should not rest with the blood examination. All the factors on table page 95 should be investigated.

Treatment.—The discovery of the cause is necessary to successful recovery. The prognosis is good if the source of hemorrhage can be controlled. If the cause be in the gastro-intestinal or genital tract and requires an operation a blood transfusion should precede it if the hemoglobin is below 50 per cent. As additional measures the diet and iron should be considered. One-quarter pound of liver, daily with green vegetables, fruit and red muscle meat stimulate cell regeneration and hemoglobin. Liver extracts are not so beneficial as the liver diet itself. Adding large amounts of iron (Blaud's 15 to 25 grains daily) to the above diet further augments hemoglobin development.[1]

Whipple has recently[2] isolated a palatable liver fraction that is 75 per cent as potent as whole liver for anemia due to hemorrhage. He finds that iron in excess of hemoglobin requirements further augments hemoglobin production. There is a similar effect by giving other metals such as copper and the mineral ash from kidney and liver.

Hemolytic Anemias.—*Acute Infections.*—Anemias occur frequently in the stormy cases of acute sepsis where there is rapid destruction of the red cells. If the infection is very severe it may inhibit the bone-marrow action. This is shown by the cases that develop anemia with lack of platelets and subnormal white cell counts. There may be absence of granules in the polymorphonuclears. This carries with it a very bad prognosis.

Anemia Due to Defective Cell Formation and Hemolysis.—It is difficult to differentiate between anemias due to blood destruction and defective cell formation. The imperfectly formed cell hemolyzes more readily than the well-formed.

Hemolytic Jaundice.—Hemolytic jaundice occurs during early adult life. The patient is more jaundiced than ill but may develop attacks of gall-stone colic late in life. The urine never contains bile and so the disease is frequently known as acholuric jaundice. The excess of urobilin in the urine and stool give them a dark color, however.

Outstanding hematological features of the disease are a slight anemia with hemoglobin rarely below 60 per cent and the color index is 1 or over. There are usually 5 to 40 per cent of reticulated red cells, the higher percentages occurring during the attacks of jaundice. The blood smear of the congenital hemolytic icterus shows microcytes which are spherical and deeply pigmented while the biconcave cells are found in the acquired type of the disease.

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[1] Murphy and Powers: The Value of Liver in the Treatment of Anemia Due to Hemorrhage, Surg., Gynec. and Obst., 1929, **47**, 480.

[2] Am. Jour. Med. Sci., 1930, **179**, 628.

Blood Picture of Typical Hemolytic Jaundice.

	Slight.	Severe.
Hemoglobin, per cent	70	50
Red blood cells:		
Million per cubic millimeter	3.8	2.2
Color index	0.9	1.3
Blood spread:		
Achromia	None	Occasional
Poikilocytosis	None	Few
Anisocytosis	Slight	Moderate
Microcytes	*Many*	*Many*
Macrocytes	None	Few
Normoblasts	None	Few
Megaloblasts	None	None
Polychromasia	Present	Present
Stippling	None	None
Reticulocytes, per cent	12	30
Icterus index (No bile in the urine)	15	50
White blood cells:		
Thousand per cubic millimeter	5	4
Differential count:		
Lymphocytes, per cent	21	30
Monocytes, per cent	10	12
Eosinophile polymorphonuclears, per cent	3	2
Neutrophile polymorphonuclears, per cent	66	51
Segmented, mature	59	42
Band nucleus, young	7	9
Myelocytes, per cent	0	3
Undetermined, per cent	0	2
Platelets, thousand per cubic millimeter	300	200

Normal red cells begin to show hemolysis to 0.45 per cent sodium chloride solution whereas in congenital and *most of the acquired cases* of hemolytic icterus, hemolysis occurs in 0.6 to 0.8 per cent saline. In any other form of icterus the resistance of the red cells is greater than normal. Because of their undue fragility the red cells are more readily destroyed by the spleen than are normal cells. *X*-ray radiation or removal of the spleen on ligation of its artery will remove the symptoms.

Addison's Anemia.—This is a severe anemia subject to remissions and accompanied by great weakness and glossitis. There is no loss of flesh and the skin has a yellowish tinge. It usually requires a deep prick to start the blood flowing, but once started it may continue for fifteen minutes due to a diminution in the blood platelets. The coagulation time is usually normal. The cells spread out discretely even on a moderately thick smear. The serum is dark in color, giving an icterus index of up to twice the normal or more. The reduction in red cells is usually greater than hemoglobin, giving a color index of over 1. Most of the cells, especially the large ones, stain very deeply. There is a preponderance of the large cells as shown by the measurement of cell diameter by the halo test or by actual measurement and charting a Price-Jones curve. Irregularity in shape is marked and nucleated forms are occasionally seen. The white cell count is normal or below normal with the polymorphonuclears diminished. The blood examination is characteristic but not conclusive.

THE BLOOD PICTURE OF TYPICAL ADDISON'S (PERNICIOUS) ANEMIA.

	Slight.	Severe.
Hemoglobin, per cent	**70**	**25**
Red blood cells:		
Million per cubic millimeter	3.0	0.8
Color index	**1.1**	**1.5**
Blood spread:		
Achromia	None	Present in small cells
Poikilocytosis	Moderate	Marked
Anisocytosis	*Moderate*	*Marked*
Microcytes	Present	Present
Macrocytes	*Present*	*Many*
Normoblasts	None	Few
Megaloblasts	None	*Few*
Polychromasia	None	Present
Stippling	None	Present
Reticulocytes, per cent	2	0
After liver diet	10	20
Icterus index	**7**	**25**
White blood cells:		
Thousand per cubic millimeter	5	2
Differential count:		
Lymphocytes, per cent	**30**	**50**
Monocytes, per cent	10	5
Eosinophile polymorphonuclears, per cent	3	1
Neutrophile polymorphonuclears, per cent	57	42
Segmented, mature	50	32
Band nucleus, young	7	10
Myelocytes, per cent	0	0
Undetermined, per cent		
Platelets, thousand per cubic millimeter	150	**50**

Free hydrochloric acid is never present in the gastric contents. The marked improvement from liver diet is of diagnostic value. On full liver diet the patient feels better in less than a week. As evidence of rapid cell regeneration reticulocytes appear in the blood on the fourth day and by the eighth day 10 to 25 per cent may be present. They then decrease in number but the hemoglobin and red cells increase; the cell increase averaging 1.5 million the first month. Sometimes an unexplained eosinophilia appears but otherwise the blood spread regains a normal appearance.

Anemia of Pregnancy.—In pregnancy there is a physiological anemia which is greatest about the eighth month. It is a relative rather than absolute anemia. It is due to increased plasma volume.

The hemoglobin may go as low as 50 per cent with the red cells about 4 million and the patient have a good prognosis. Figures lower than these should be considered pathological. The increased plasma may be regarded as nutrition medium for the growing fetus. During the last few weeks of pregnancy the fetus is more active and requires more oxygen so the hemoglobin increases. The hemoglobin should return to at least 75 per cent two weeks after confinement. Anemia present later than two weeks after confinement indicates excessive hemorrhage, sepsis, chlorotic or pernicious anemia.

In pregnancy, anemia is frequently confused with toxemia, postpartum infection or miliary tuberculosis. Because of the hemic murmur and tachycardia of anemia, endocarditis is frequently suspected. In toxemia the blood-pressure is always elevated whereas it is usually low in anemias. An examination of the hemoglobin, and red cells is necessary. The anemias have a very definite hemoglobin and red cell deficiency, the red cell count being frequently below 1 million. About one-half the patients have a blood picture identical with Addison's anemia and the other half a blood picture of the secondary achromic anemia. The anemia usually makes its appearance in the latter months of pregnancy or in the puerperium. Premature labor may occur with little or no blood loss. The presence of anemia is definite indication for abortion and transfusion. If left to run its course the mortality is high and liver diet is only of value where the blood picture is like Addison's anemia. The disease does not recur in subsequent pregnancies.

Anemia Due to Intestinal Parasites.—Fish tapeworm and the hookworm may cause severe anemia. Less than 1 per cent of those infected with the fish tapeworm develop anemia which usually has a blood picture resembling pernicious anemia but at times there is free hydrochloric acid in the stomach. There may be an eosinophilia. The anemia usually disappears after the parasite is expelled. Hookworm disease produces a posthemorrhagic type of anemia and eosinophilia may be present. When fish tapeworm anemia has a macrocytic hyperchromic blood picture like Addison's anemia, liver diet will bring the blood back to normal. Suspecting a tapeworm in anemia patients, liable to such infection and an examination of the stool for ova is therefore very important.

Anemia Due to Defective Cell Formation.—Anemia of Malnutrition.—Anemia of malnutrition is of great importance. It may be due either to insufficient nutrition or a poorly balanced diet, usually with a lack of red meats. It is the important factor in many of the anemias which are apparently due to a hemorrhage.

If the body is well nourished, the bone-marrow can reproduce in a remarkably short time the cells lost through bleeding. If the body is poorly nourished at the time of the hemorrhage, good feeding afterward will induce a slower return and at times it will not induce any improvement, the bone-marrow apparently being in such an exhausted state that it cannot readily turn the good nutrition into cells. The same phenomenon is true to a lesser extent regarding the effect of nutrition on anemias due to bacterial toxins.

General hygienic conditions such as fresh air, sunlight, cleanliness and exercise, while important are secondary to nutrition. A careful history of what the patient's meals consist of will detect errors in the diet. A proper knowledge of caloric and vitamin requirements will enable the physician to supply the patient with a written outline of what he should eat.

There is a great iron deficit in the blood which has a cell picture of the chlorotic type.

Chlorotic Anemia.—*Classical chlorosis* is a rare disease occurring in girls, usually at or after puberty. The pallor is extreme, there is no loss of flesh and the blood picture is one of a very marked secondary anemia with the hemoglobin 50 per cent or lower while the red cells may be 4 million. The achromia is so marked that the cells appear white except for their narrow stained border. The irregularity in size and shape is slight and microcytes predominate. Normoblasts may be present. The serum is also pale giving a color index below 4. Liver diet has no beneficial effect but large doses of iron (Blaud's), rest and a well-balanced diet including green vegetables induce a quick recovery.

Chronic chlorotic anemia occurs in older women and even in men. It is really a chronic secondary anemia having the hemoglobin much more decreased than the red cells. No definite cause can be found for it. There is probably a lack of iron in the diet or for some reason the cells cannot make up hemoglobin. It may persist for years or remissions may occur. In the past it differed from the classical chlorosis in that it did not show the usual improvement under rest, diet and iron treatment but Mills[1] has shown that anemic patients having a low hemoglobin but a normal cell count recover very rapidly when given 3 grains of iron and $\frac{1}{50}$ grain of copper thrice daily. The improvement is almost as spectacular as that of pernicious anemia when treated on liver diet. The recovery of experimental animals having anemia is more rapid when spinach solution is given with milk diet than when an iron-copper or even an iron,

[1] Canadian Med. Assn. Jour., 1930, **22**, 175.

copper and manganese mixture is given with the milk[1] As well as iron, copper and manganese, spinach contains aluminium, antimony, calcium, magnesium, sodium, potassium, tin and zinc. This would support the view held by Whipple that not only iron but a group of inorganic minerals are active in hemoglobin building.

Blood Picture of Typical Chlorotic Anemia.

	Slight.	Severe.
Hemoglobin, per cent	55	25
Red blood cells:		
Million per cubic millimeter	4.2	3.5
Color index	0.65	0.36
Blood spread:		
Achromia	*Marked*	*Very marked*
Poikilocytosis	None	Slight
Anisocytosis	None	Moderate
Microcytes	*Few*	*Many*
Macrocytes	*None*	*Few*
Normoblasts	None	Few
Megaloblasts	None	None
Polychromasia	None	None
Stippling	None	None
Reticulocytes, per cent	0 to 3	0 to 3
Icterus index	3	2
White blood cells:		
Thousand per cubic millimeter	5	4
Differential count:		
Lymphocytes, per cent	30	35
Monocytes, per cent	10	10
Eosinophile polymorphonuclears, per cent	2	2
Neutrophile polymorphonuclears, per cent	58	54
Segmented, mature	48	42
Band nucleus, young	10	12
Myelocytes, per cent	0	0
Undetermined, per cent	0	0
Platelets, thousand per cubic millimeter	500	500

Toxic Anemia.—Chronic infections especially subject to anemia are those where the products of infection are retained. Sinus or pleural empyema, abscess of teeth and subphrenic, perinephritic, pelvic or prostatic abscess are examples.

The only characteristic blood findings are in the white cells. Leucocytosis is present in active but not in long-standing cases. There is a definite increase, over 10 per cent, in the percentage of polymorphonuclears with band nuclei. These young forms represent an increased supply to combat the infection. The polymorphonuclears have deep staining granules. In subacute bacterial endocarditis there is usually a leukopenia with an increase in staff-shaped nuclear forms of polymorphonuclears and an increase in the endothelial cells.

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[1] Mitchell and Miller: Jour. Biol. Chem., 1929, **85**, 355.

The hemoglobin and red cells show a considerable decrease, making the color index often but not always below 1. The percentage of reticulocytes will determine bone-marrow activity which is never very high in toxic anemia. In the smear the red cells show a moderate anisocytosis. There may be a marked achromia or many of the larger cells may be hyperchromic, and some show fragmentation.

A Blood Picture of Toxic Anemia from Chronic Infection.

	Slight.	Severe.
Hemoglobin, per cent	65	30
Red blood cells:		
Million per cubic millimeter	4	3
Color index	0.8	0.5
Blood spread:		
Achromia	Slight	Marked
Poikilocytosis	None	Slight
Anisocytosis	Slight	Marked
Microcytes	Present	Present
Macrocytes	None	Present
Normoblasts	None	None
Megaloblasts	None	None
Polychromasia	None	Occasional
Stippling	None	None
	(except in lead poisoning)	
Reticulocytes, per cent	2	0
Icterus index	4	2
White blood cells:		
Thousand per cubic millimeter	7	4
Differential count:		
Lymphocytes, per cent	25	28
Monocytes, per cent	10	12
Eosinophile polymorphonuclears, per cent	2	3
Neutrophile polymorphonuclears, per cent	60	39
Segmented, mature	48	28
Band nucleus, young	12	15
Myelocytes, per cent	0	1
Undetermined, per cent	3	3
Platelets, thousand per cubic millimeter	300	200

Because toxic anemias have no characteristic blood pictures it requires a systematic and painstaking search for the cause. If discovered the removal of the cause together with the treatment outlined for hemorrhagic anemia results in improvement provided the damage to the bone-marrow is not too severe.

Malignancy.—Carcinoma of the stomach and large bowel are the commonest types of cancer to produce anemia. Cancer of the esophagus or lungs or sarcoma frequently do not produce anemias. The anemia frequently appears when the tumor ulcerates. It is probably caused by the production of a toxin as well as the oozing of blood from the ulcer. The blood serum is pale if the tumor does not involve the bile duct. In the blood picture the color index is usually below 1 per cent. Irregularity in the size of the cells is seen and if there is no bleeding, macrocytes may be present. Sometimes normoblasts are present. Blood platelets are often increased. Metastases into bone-marrow may release into the peripheral blood primitive cells, normoblasts, megaloblasts or myelocytes so that at times if the primary growth is obscure and no *x*-rays of the osseous system are taken one may be misled into a diagnosis of myeloid leukemia. There is frequently a leucocytosis, which is sometimes very high and the percentage of polymorphonuclears is increased.

Chronic Nephritis.—In chronic nephritis the anemia is of prognostic value. Nephritics having a hemoglobin below 50 per cent or a red cell count below 3 million rarely live a year. The blood-pressure is always elevated in the anemia of chronic nephritis. The blood picture is of the achromic type, the color index is usually under 1 and there is frequently a leucocytosis.

Myxedema.—Either myxedema or hyperthyroidism may be a cause of anemia. The blood picture is usually of the chlorotic type but may approach that of Addison's anemia. At times diabetes or Addison's disease may be associated with a severe anemia.

Lead Poisoning.—The anemia is not severe in lead poisoning although the patient appears very pale. It is chlorotic in type. The hemoglobin rarely goes below 50 per cent or the red cells below 3.5 million. The basophilic granules when present in an anemia of no more severe grade than this are diagnostic of lead poisoning. (See page 41.) Albuminuria is a common finding in lead poisoning.

Aplastic Anemia.—Aplastic anemia shows itself clinically as an anemia, an infection or recurring hemorrhages and is due to exhausting hemorrhage or sepsis, *x*-ray exposure, arsphenamin, trinitrotoluene or benzol poisoning. Benzol is an excellent rubber solvent and is much used in manufacturing. Giving too much arsphenamin to an asthenic patient may precipitate an aplastic anemia. Repeated exposures to *x*-ray radiation will cause an aplastic anemia. Radium produces the same effect as *x*-ray radiation but ordinarily the contact with this substance is too slight to do any harm. It is a wise precaution for all engaged in *x*-ray work to have monthly blood examinations, although modern precautions are usually satisfactory in preventing bad effects from the rays. A single dose of the usual deep *x*-ray therapy will produce a slight polymorphonuclear leucocytosis in forty-eight hours, followed by a leukopenia, chiefly in the lymphocytes, which reaches its maximum in a week and returns to normal in a month. If the blood is up to normal to begin with, there is but little danger of developing an aplasia. Erythema doses of *x*-ray produce a lymphocytosis and increased platelet count.

Frequently the advanced states of Addison's anemia, pernicious anemia of pregnancy or anemia from fish tapeworm, are hypoplastic but these patients respond to liver diet.

All elements from the bone-marrow are diminished. The earliest sign is a leukopenia and the percentage of polymorphonuclears is very much decreased. The leukocyte count may fall below 1000 with less than 30 per cent of polymorphonuclears. Later the red cells decrease and may show fragmentation together with irregularity of size and shape. *No reticulated forms are found.* The red cell count may fall to 1,000,000 and the platelets be less than 20,000 so that none can be seen on the blood spread. The cause should be sought and removed if possible. A well-balanced diet, large doses of iron and blood transfusion are indicated. The prognosis is usually bad.

The Blood Picture of Typical Aplastic Anemia.

	Slight.	Severe.
Hemoglobin, per cent	55	30
Red blood cells:		
Million per cubic millimeter	3.8	1.2
Color index	0.99	1.2
Blood spread:		
Achromia	Occasional	Occasional
Poikilocytosis	Slight	Slight
Anisocytosis	None	Frequent
Microcytes	None	Few
Macrocytes	None	Few
Normoblasts	*None*	*None*
Megaloblasts	None	None
Polychromasia	None	None
Stippling	None	None
Reticulocytes, per cent	**0**	**0**
Icterus index	4	2
White blood cells:		
Thousand per cubic millimeter	**2.5**	**0.8**
Differential count:		
Lymphocytes, per cent	42	64
Monocytes, per cent	7	11
Eosinophile polymorphonuclears, per cent	2	0
Neutrophile polymorphonuclears, per cent	49	25
Segmented, mature	47	25
Band nucleus, young	**2**	**0**
Myelocytes, per cent		
Undetermined, per cent		
Platelets, thousand per cubic millimeter	**120**	**14**

Myelophthisic Anemia.—Myelophthisic anemia is a term applied to an aplastic anemia caused by the invasion of the bone-marrow by carcinoma or hypernephroma metastases or the leukemias. There is very little relation between the amount of marrow invaded and the degree of anemia. A patient having myeloma with extensive bone invasion may have blood near normal while in another a few secondary nodules in the pelvis may have advanced aplastic anemia. There is a short period of bone-marrow stimulation followed by a progressive aplastic anemia. Lee and Minot describe a type of aplastic anemia where the bone-marrow is normal and the blood state most likely due to a failure of the bone-marrow to cast off the cells.

Leukemia.—The anemia occurring in the leukemias is a very definite one with low hemoglobin, reduced red cell count, a blood picture showing marked achromia and frequently nucleated red cells. This serves to differentiate leukemia from the benign mononucleosis or glandular fever in which no anemia occurs.

Banti's Disease (Splenic Anemia).—A chlorotic anemia associated with splenic enlargement are the outstanding findings. Later portal cirrhosis of the liver, jaundice, ascites and hemorrhages occur. From unknown cause, probably toxic, a fibrosis starts around the central arterioles of the spleen and gradually replace all the normal splenic pulp. This syndrome was described by Banti in 1894 and included such diseases as thrombosis of the splenic vessels and syphilis, which are associated with splenomegaly and chlorotic anemia. For this reason a diagnosis of Banti's disease is today only made by excluding all other diseases associated with splenomegaly and chlorotic anemia such as:

Gaucher's splenomegaly.
Biliary cirrhosis.
Visceral syphilis.
Chronic malaria.
Hemolytic jaundice.
Pernicious anemia (in remission).
Aleukemic leukemia.
Atypical Hodgkin's disease.

Sickle-cell Anemia.—Sickle-cell anemia is found in negroes. During the attacks the patients have a marked anemia, enlarged liver, edema of the lower limbs and probably ulcers. The red cell resistance and bilirubin content of blood is increased. There is an increase of urobilin in the urine but no bile. If a drop of blood be placed on a glass slide, covered with a cover-glass and its margin ringed with vaseline the cells in less than twenty-four hours take on a crescentic or linear shape. The cause is unknown and splenectomy is under consideration as a remedy.

Anemia of Childhood.—Anemias of children are usually associated with splenic enlargement and are called splenic anemia, rachitic anemia or anemia pseudo-leukemia infantum of von

Jaksch. In von Jaksch's disease the blood picture is that of severe Addison's anemia with a leucocyte increase to 20,000, the polymorphonuclears predominating. It likely included cases of leukemia. Erythroblastic anemia, which describes the cellular reaction, is a more suitable term. The blood picture is that of a severe Addison's anemia during a crisis when many unripe forms are in the blood. According to Faber, liver diet produces a quick recovery. Repeated blood examinations will make certain of early leukemia.

A very definite chlorotic anemia frequently occurs from poor feeding, rickets, infantile scurvy (Barlow's disease) or infections such as tuberculosis, lymph adenitis or syphilis. Diets poor in iron and vitamin content given to a constitutionally weak child are very common causes of anemia. Proper diet and hygiene will usually produce striking therapeutic results. Anemia in children shows more bone-marrow activity than in adults. For this reason very marked cell changes do not carry such a serious prognosis as the same finding would in adults.

LEUKEMIA.

Leukemia is a hyperplasia from unknown cause, of the leukoblastic tissues, either in the bone-marrow or lymph glands. Many immature white cells usually appear in the peripheral blood.

TYPES OF LEUKEMIA AND ALLIED DISEASES.

- **Chronic lymphatic leukemia.**
- **Chronic myeloid leukemia.**
- **Acute leukemia.**
- **Aleukemic leukemia.**
- **Lymphosarcoma.**
- **Hodgkin's disease.**
- **Glandular fever.**
- **Agranulocytic angina.**

The leukemias, lymphosarcoma and lymphogranuloma (Hodgkin's disease) are diseases with a fatal termination. Localized glandular enlargement usually starting in the neck characterizes lymphosarcoma and lymphogranuloma. In the acute forms of leukemias there is a slight enlargement of ALL the lymph glands.

Chronic Lymphatic Leukemia.—The patient complains of the symptoms associated with severe anemia such as weakness, dizziness or loss of weight. He may notice an enlarged lymph gland, tonsil or abdominal enlargement due to the spleen, which however is not as large as in myeloid leukemia. There are periods of fever and skin disorders may be present.

Blood examination shows a rapid decrease from normal in the hemoglobin and red cells but the white cells are from 50,000 to 200,000 and composed of over 90 per cent lymphocytes which show no granules with peroxidase stains. These cells rupture easily producing the irregular bluish masses. The disease runs its fatal course in two to ten years. *X*-ray or radium treatment over the enlarged glands temporarily lowers the blood count and gives an extra year or two of life.

Blood Picture of Typical Chronic Lymphatic Leukemia.

	Early.	Late.
Hemoglobin, per cent	**60**	**20**
Red blood cells:		
Million per cubic millimeter	**3.2**	1.2
Color index	0.9	0.8
Blood spread:		
Achromia	Definite	Marked
Poikilocytosis	Slight	Marked
Anisocytosis	Slight	Marked
Microcytes	Slight	Marked
Macrocytes	Few	Few
Normoblasts	None	Present
Megaloblasts	None	None
Polychromasia	None	Present
Stippling	None	Present
White blood cells:		
Thousand per cubic millimeter	**75**	**200**
Differential count:		
Lymphocytes, per cent	**90**	**95**
Monocytes, per cent	4	2
Eosinophile polymorphonuclears, per cent	0	0
Neutrophile polymorphonuclears, per cent	6	0
Segmented, mature	3	
Band nucleus, young	3	
Myelocytes, per cent	0	1
Undetermined, per cent	0	2
Platelets, thousand per cubic millimeter	150	50

Chronic Myeloid Leukemia.—Clinically the most outstanding feature of this disease is weakness and abdominal enlargement from the enormous spleen although a few cases have only a moderate enlargement of the spleen. The blood examination shows a severe anemia and 50,000 to 800,000 white cells. The polymorphonuclears and myelocytes form 90 per cent of the total white cells. Some of the myelocytes are much more granular than others as seen by the oxidase reaction. The average length of life after diagnosis is three years. A few patients have lived ten years. *X*-ray or radium treatment lowers the white cell count, makes the patient more comfortable and probably adds two or three years to his life.

BLOOD PICTURE OF TYPICAL CHRONIC MYELOID LEUKEMIA.

	Early.	Late.
Hemoglobin, per cent	**50**	**20**
Red blood cells:		
Million per cubic millimeter	**3**	**1.5**
Color index	0.8	0.6
Blood spread:		
Achromia	Moderate	Marked
Poikilocytosis	Present	Present
Anisocytosis	Slight	Moderate
Microcytes	Present	Present
Macrocytes	None	Many
Normoblasts	*Present*	*Many*
Megaloblasts	None	Few
Polychromasia	Present	Present
Stippling	Slight	Marked
White blood cells:		
Thousand per cubic millimeter	**50**	**1000**
Differential count:		
Lymphocytes, per cent	7	3
Monocytes, per cent	3	1
Eosinophile polymorphonuclears, per cent	6	2
Neutrophile polymorphonuclears, per cent	54	30
Segmented, mature	28	12
Band nucleus, young	26	18
Basophiles	45	3
Myelocytes, per cent	**22**	**56**
Undetermined, per cent	4	6
Platelets, thousand per cubic millimeter	400	10 to 600

Acute Leukemia.—Following some focal infection, which may not be severe, the patient becomes very ill with *general* glandular enlargement, fever and often hemorrhages. Death occurs in one to two

BLOOD PICTURE OF TYPICAL ACUTE LYMPHATIC LEUKEMIA.

	Early.	Severe.
Hemoglobin, per cent	**50**	**20**
Red blood cells:		
Million per cubic millimeter	**2**	**0.7**
Color index	1.2	1.4
Blood spread:		
Achromia	Present	Present
Poikilocytosis	Present	Present
Anisocytosis	Present	Present
Microcytes	Present	Present
Macrocytes	None	Present
Normoblasts	Present	Present
Megaloblasts	None	Present
Polychromasia		
White blood cells:		
Thousand per cubic millimeter	**10**	**100**
Differential count:		
Lymphocytes, per cent	**90**	**95**
Monocytes, per cent	3	3
Eosinophile polymorphonuclears, per cent	0	0
Neutrophile polymorphonuclears, per cent	3	2
Myelocytes, per cent	2	0
Undetermined, per cent	1	
Platelets, thousand per cubic millimeter	150	20

weeks. The stained blood smear shows increased numbers of abnormal mononuclear cells, which may be so immature as to defy classification. Smears stained with the peroxidase reaction as well as ordinary stains will usually differentiate *myeloblastic* cells with their cytoplasmic granules from the *lymphatic* cells which have none.

Acute lymphatic leukemia occurs in young people and is characterized clinically by fever, pallor and asthenia. The lymph glands are enlarged all over the body and the spleen, although not so enlarged as in the myeloblastic form, is palpable. There is usually a sore throat with fever, and hemorrhages may be present.

The white cells are increased to 20,000 or 30,000 or more, lymphocytes making up over 95 per cent in the differential count.

Anemia is present and is a most valuable point in differentiating acute leukemia from infections such as a pertussis or measles which may have a white cell picture resembling leukemia.

Acute myeloblastic leukemia clinically resembles the acute lymphatic except that there is more splenic enlargement. Examination of the blood film shows a profound anemia and many white cells which at first appear as large immature lymphocytes but the peroxidase stain will bring out some granularity of the cytoplasm. Over 30 per cent of these cells indicate a myeloid origin of the leukemia.

BLOOD PICTURE OF TYPICAL ACUTE MYELOBLASTIC LEUKEMIA.

	Early.	Severe.
Hemoglobin, per cent	**55**	**25**
Red blood cells:		
Million per cubic millimeter	**2.5**	**1**
Color index	1.1	1.2
Blood spread:		
Achromia	Present	Present
Poikilocytosis	Present	Present
Anisocytosis	Present	Present
Microcytes	Present	Present
Macrocytes	None	None
Normoblasts	*Present*	*Present*
Megaloblasts	None	None
Polychromasia	Present	Present
Stippling	Present	Present
White blood cells:		
Thousand per cubic millimeter	**15**	**60**
Differential count:		
Lymphocytes, per cent	5	4
Monocytes, per cent	2	2
Eosinophile polymorphonuclears, per cent	3	5
Neutrophile polymorphonuclears, per cent	**29**	**14**
Segmented, mature	11	6
Band nucleus, young	**18**	**8**
Myelocytes, per cent	**16**	**13**
Myeloblasts (premyelocytes), per cent	**38**	**57**
Undetermined, per cent	7	5
Platelets, thousand per cubic millimeter	450	10 to 700

Aleukemic Leukemia.—This condition is quite difficult to diagnose. The patient is very weak, pale and may have hemorrhages, spontaneously or with slight injuries. There is usually splenic or glandular enlargement. The total white cells are not increased and there may even be a leukopenia. *There is usually a marked disturbance in the differential leucocyte count.* Most of the cases of aleukemic leukemia are lymphatic and here the lymphocytes will form 90 per cent of the white cells. In the myelogenous type the lymphocytes may form even less than 10 per cent polymorphonuclears and myelocytes, many eosinophilic and basophilic make up the remainder. A few myeloblasts may be present. The disease is rapidly fatal, death occurring in a few weeks to a few months. There are usually severe hemorrhages just before death. At times the aleukemic type develops into the usual form with great numbers of white cells in the blood.

BLOOD PICTURE OF TYPICAL ALEUKEMIC LEUKEMIA.

	Early.	Severe.
Hemoglobin, per cent	**60**	**20**
Red blood cells:		
Million per cubic millimeter	**2.8**	**0.06**
Color index	1.0	1.6
Blood spread:		
Achromia	Present	Present
Poikilocytosis	Present	Present
Anisocytosis	Present	Present
Microcytes	Present	Present
Macrocytes	None	None
Normoblasts	Present	Present
Megaloblasts	None	None
Polychromasia	Present	Present
Stippling	Present	Present
White blood cells:		
Thousand per cubic millimeter	**3**	**10**
Differential count:		
Lymphocytes, per cent	**90**	**95**
Monocytes, per cent	3	2
Eosinophile polymorphonuclears, per cent	0	0
Neutrophile polymorphonuclears, per cent	4	2
Myelocytes, per cent	2	1
Undetermined, per cent	1	0
Platelets, thousand per cubic millimeter	200	100

ALEUKEMIC LEUKEMIA.	SEPTIC INFECTION.
1. Severe ulceration in the mouth.	1. May be slight ulcers.
2. Sterile blood cultures.	2. Positive blood cultures.
3. Lymphocytic increase in blood.	3. Polymorphonuclear increase in blood.
4. General glandular enlargement.	4. Glandular enlargement localized to the infected area.

Lymphosarcoma.—The mediastinal, cervical or retroperitoneal group of glands are more frequently involved as a localized enlargement. There is little or no enlargement of the spleen. The mediastinal glands enlarge rapidly and produce pressure symptoms if they are at the back of the thorax and physical signs if they are at the front. There is secondary anemia with no change in the white cells although occasionally typical cases of lymphosarcoma have developed into lymphatic leukemia. It is frequently confused with lymphoblastoma (Hodgkin's disease), but the *x*-ray aids in the diagnosis. Under *x*-ray treatment lymphosarcoma will disappear in less than a week, if it be of the primitive lymphocytic type. The large reticulo-endothelial cell type is very resistant to *x*-ray. Lymphoblastoma shrinks to about one-half its size in several weeks. Carcinomatous tumors are little affected.

Hodgkin's Disease (Lymphoblastoma, Lymphogranuloma).—This is a localized enlargement of lymphatic glands, usually in the cervical region. The enlargement is due to proliferation of an endothelial cell about twice the size of the lymphocyte and is probably caused by the avian tubercle bacillus. The patient usually has a secondary anemia with a leucocytosis up to 20,000 in the afebrile stage. In the afebrile stage the monocytes may be double their normal number but as this also occurs in other chronic glandular diseases it is of no value in differential diagnosis. Response to *x*-ray or microscopic examination of a section of enlarged glands are valuable in diagnosis.

BLOOD PICTURE OF TYPICAL GLANDULAR FEVER.

	Slight.	Severe.
Hemoglobin, per cent	**100**	**60**
Red blood cells:		
Million per cubic millimeter	**5.2**	**4**
Color index	0.9	0.75
Blood spread:		
Achromia	None	None
Poikilocytosis	None	None
Anisocytosis	None	None
Microcytes	None	None
Macrocytes	None	None
Normoblasts	None	None
Megaloblasts	None	None
Polychromasia	None	None
Stippling	None	None
White blood cells:		
Thousand per cubic millimeter	**15**	**40**
Differential count:		
Lymphocytes, per cent	**70**	**85**
Monocytes, per cent	10	8
Eosinophile polymorphonuclears, per cent	0	0
Neutrophile polymorphonuclears, per cent	14	5
Segmented, mature	9	3
Band nucleus, young	5	2
Myelocytes, per cent	2	0
Undetermined, per cent	4	2
Platelets, thousand per cubic millimeter	250	200

Glandular Fever. (Acute Infectious Mononucleosis.)—This is a benign disease which has some of the blood findings of lymphatic leukemia. The patient has a fever varying from 100° to 102° F., general glandular enlargement and from 15,000 to 30,000 leucocytes per cu.mm., over 80 per cent of which are lymphocytes. The most valuable diagnostic point is that there is no anemia with this condition in distinction to the marked anemia of leukemia.

Agranulocytic Angina.—Clinically this disease is characterized by foul, necrotic ulceration in the mouth or throat. Smears of the ulcers show the bacillus fusiformis and spirillum of Vincent. Few polymorphonuclear neutrophile cells leave the bone-marrow and those in the blood show a diminution of granularity in their cytoplasm. Agents destructive to bone such as exhausting hemorrhage, hypervirulent infection, benzol, arsphenamin or trinitrotoluene (T.N.T.) poisoning, and prolonged or repeated exposure to *x*-ray will produce a somewhat similar state of the white cells.

Blood Picture of Typical Agranulocytic Angina.

	Slight.	Severe.
Hemoglobin, per cent	50	15
Red blood cells:		
Million per cubic millimeter	3	1.8
Color index	0.8	0.4
Blood spread:		
Achromia	None	Present
Poikilocytosis	None	Marked
Anisocytosis	None	Marked
Microcytes	None	Present
Macrocytes	None	Few
Normoblasts	None	None
Megaloblasts	None	None
Polychromasia	None	None
Stippling	None	None
White blood cells:		
Thousand per cubic millimeter	4	1.5
Differential count:		
Lymphocytes, per cent	36	52
Monocytes, per cent	19	38
Eosinophile polymorphonuclear, per cent	2	0
Neutrophile polymorphonuclear, per cent	38	10
Segmented, mature	13	2
Band nucleus, young	25	8
Myelocytes, per cent	0	0
Undetermined, per cent	5	0
Platelets, thousand per cubic millimeter	124	35

No neutrophile granules in the polymorphonuclear cells.

Agranulocytic angina is usually fatal. There is a closely allied state having necrotic ulceration in the throat due to Vincent's infection and over 80 per cent monocytes in the blood, which is always fatal. This is in marked contrast to the good prognosis in streptococcal sore throat, which, although it may cause peritonsillar abscess produces little if any superficial necrosis and is always associated with a polymorphonuclear leucocytosis.

RECENT VIEWS ON HEMATOLOGY.

(From the publications of Sabin, Doan, Zerfas, Cunningham and others.)

The bone-marrow is the source of the red and granular white cells and blood platelets in adult life. It is therefore of the utmost importance in disorders of these cells.

Few realize that the total volume of the bone-marrow is almost as large as the liver. Before fifteen years of age the marrow of all bones is engaged in cell production. After fifteen years only the spongy flat bones of the body and the heads of the femur and humerus produce them. If there is a call for more cells the field of red-cell-forming activity spreads downward in the extremities until in very severe anemias all the bone-marrow to the hands and feet may be red due to the red cell production.

Microscopically the red cells originate in small collapsed capillaries connecting capillaries through which the blood flows. From the endothelial lining of these collapsed capillaries, megaloblasts arise and divide. After a number develop they turn to normoblasts and finally to erythrocytes. Now the capillary opens and the cells float out into the blood stream while other capillaries close and take on the process of cell formation. With an increased demand, immature, reticulated cells, normoblasts and in cases of extreme need, megaloblasts appear in the blood stream.

The structure of the normal red cell is a biconcave or concavo-convex disk with a framework of lipoid and hemoglobin firmly bound to the stroma of the cell. Immature forms when specially stained present polychromasia, reticulation and stippling due to the greater permeability or defect in the cell stroma. The function of the red cell is to carry oxygen to the tissues by means of its hemoglobin. The cells have a large surface and can take much oxygen. Each gram of hemoglobin can combine with 1.34 cc. of oxygen.

After several months of service the red cells break up by first putting out a long process and becoming a poikilocyte. This process finally breaks off and is taken up by one of the reticulo-endothelial cells which in great numbers line the capillaries of the bone-marrow, in the spleen, the liver and the lymph nodes. Here the blood pigments are converted into bile pigments, chiefly bilirubin, which as it passes into the bile ducts of the liver becomes bile and if there is no free outlet into the intestine it is reabsorbed into the general circulation, causing jaundice and bile in the urine. The bilirubin before separated from the plasma by the liver does not produce bile in the urine.

Bacteria in the intestinal tract change the bile to urobilinogen which is absorbed into the circulation to be re-excreted by the liver. When an excess is present it enters the general circulation and because the liver has a urobilin threshold, is excreted by the urine. Liver disease raises the threshold. Thus an excess of urobilinogen signifies excessive blood destruction or damaged liver. If the bile flow into the intestine is entirely obstructed, there will be *no* urobilinogen formation and so the urine will contain bile but no urobilin. This state is present in the complete obstruction due to new growth involving the common bile duct.

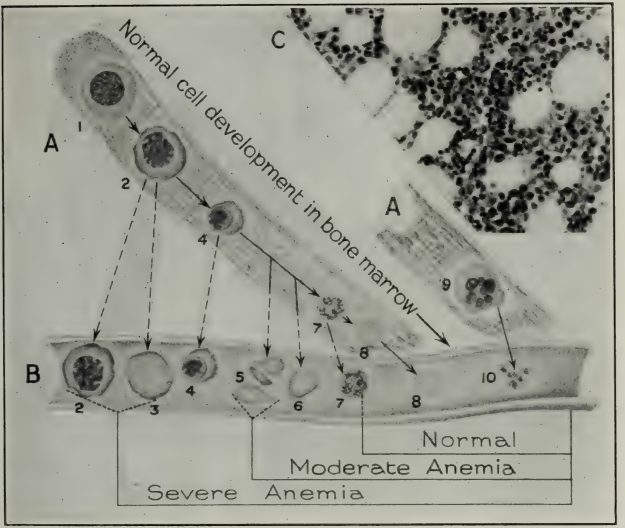

FIG. 25.—Diagram illustrating the steps of red cell development in bone-marrow and the conditions under which they are called out into the blood stream.

A. Bone-marrow of rib.
B. Peripheral blood stream.
1. Endothelial cell lining red cell forming sinus in bone-marrow.
2. Megaloblast.
3. Megalocyte or macrocyte.
4. Normoblast.
5. Poikilocytes resulting from imperfect formation or action of toxins on cells.
6. Hypochromic cell low in hemoglobin as result of more rapid cell formation than hemoglobin synthesis. This occurs after hemorrhage in hemoglobin dyscrasia or where the diet is deficient in iron.
7. Reticulated red cell (reticulocyte).
8. Normal red cell.
9. Megakarocyte, the bone-marrow giant-cell from whose cytoplasm particles separate to form blood platelets.
10. Blood platelets.
C. Photomicrograph × 200 of red cell forming sinus-capillary in bone-marrow.

Bone-marrow Studies in Addison's Anemia.—The bone-marrow of the tibia has been studied during life in cases of Addison's anemia by Dr. Peabody. He has found great megaloblastic proliferation during the attacks. These started to return to normal earlier than the blood count came back to normal. He also noted that in hemolytic jaundice where the greatest cell hemolysis occurs the bone-marrow was almost normal. This led him to think that pernicious anemia is probably a malignant activity of the bone-marrow in which many immature cells are thrown into the blood stream, rather than a pure hemolytic blood disease, probably the result of a chronic infection in the upper gastro-intestinal tract producing a toxin which acts on the blood. The change in the blood is largely the result, rather than the cause of abnormal bone-marrow function. Numerous clasmatocytes and other monocytes are seen in the bone-marrow ingesting red blood cells.

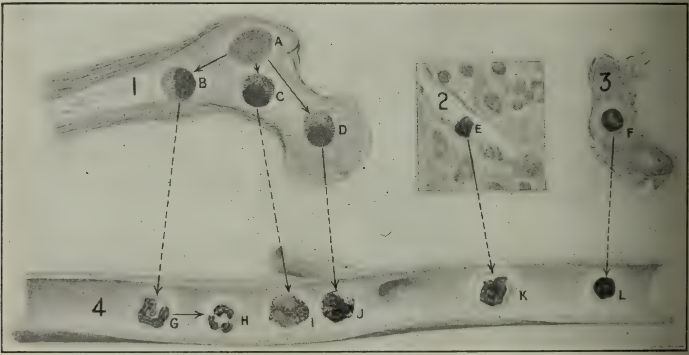

Fig. 26.—Diagram illustrating development of white blood cells.

1. *Bone-marrow.*

A. Myeloblast
Myelocytes
B. Neutrophile.
C. Eosinophile.
D. Basophile.

2. *Bone-marrow.*
Capillary × 500.

E. Endothelial phagocyte breaking from capillary wall.

3. *Lymph gland.*

F. Lymphoid cell.

4. *Blood stream.*

G. Neutrophile polymorphonuclear solid nucleus (young form).
H. Neutrophile polymorphonuclear segmented nucleus (mature).
I. Eosinophile polymorphonuclear.
J. Basophil polymorphonuclear.
K. Monocyte.
L. Lymphocyte.

The White Blood Cells.—The leucocytes in extra-uterine life consist of:

1. Granular cells from the myeloid portion of bone-marrow.
2. Monocytes from the reticulo-endothelial cells in the bloodvessel walls chiefly of the spleen, liver and lymph sinuses.
3. Lymphocytes from lymph glands.

The polymorphonuclear cells arise from the myeloid areas which are beside large capillaries mainly at the margin of the bone-marrow, and are separate from the red-cell-forming areas. The granular leucocytes originate from a reticular cell just outside the capillary and when developed through the slightly granular myeloblasts and very granular myelocyte stage into the polymorphonuclear they enter the capillary by amœboid movement.

The origin of the monocytes is not definitely agreed upon. Likely this type of cell consists of several different types. Some are very active, others are motionless. The lymphocytes are identical with the main cells in the lymph glands and spleen and the enormous increase in the blood of a leukemic patient is attended by lymphatic glandular and splenic enlargement. It has been very difficult to obtain direct evidence of the entry of lymph gland cells into the blood stream but, through the lymph vessels by way of the thoracic duct is the most likely mode of entrance.

Function of the White Cells.—The polymorphonuclear neutrophiles are actively phagocytic toward bacteria and all foreign matter and they also digest the material ingested. They probably aid in carrying nutrition to the tissues. Some of the monocytes are actively phagocytic but others are not. They increase by proliferation of the reticulo-endothelial system when bacteria or insoluble particles are injected into the blood stream. No active function has been ascribed to the lymphocytes. They increase in bacillary infections. They have a clear cytoplasm and no phagocytic power is evident. They probably aid in intestinal absorption, produce antibodies and "fix" toxins.

Information Obtained by Supravital Staining.—This method consists in allowing a very thin film of neutral red to dry on a glass slide, spreading out a blood film over it and covering with a cover-glass while the film is still wet and sealing the margins of the coverslip with paraffin to prevent drying out of the film. If the film be placed on a warm stage of a microscope kept at body temperature, oil-immersion observation will reveal:

(*a*) Most of the polymorphonuclears take on amœboid movement.

(*b*) Most of the monocytes take on amœboid movement and ingest particles of the red stain into their cytoplasm.

(*c*) The lymphocytes are motionless.

The amœboid movement of the polymorphonuclears begins by the cell extending part of its outline in one direction through which the granules flow and later the cell all moves up to the point to which the projection has extended.

Because some of the polymorphonuclears are motionless they are assumed to be dead. They die off in showers, usually at hourly intervals, and they are promptly replaced. It is estimated that one-fifth of the total die off in twenty-four hours. There is a quicker and smaller rhythm variation for the lymphocytes. The only exit for the large numbers of dead and dying leucocytes is through the mucosa into the alimentary canal.

Isaacs has demonstrated that a cell count of the undiluted saliva is a good index to the rate of production of white cells. Five to 150 per cu.mm. are normal. There are thousands per cu.mm. when the bone-marrow is active and the cells are rapidly destroyed. Marked disturbance of the erythropoietic system (anemia) occurs in diseases of the leukopoietic system. Diseases of the erythropoietic system only slightly disturb the leukopoietic system.

Blood Platelets.—Blood platelets are small masses of cytoplasm from the *megakaryocytes*, which are large cells found immediately on the outside of capillary walls. The cytoplasm has amœboid movement by which it works its way into the circulation and breaks up to form blood platelets. When bone-marrow recovers from any injury the platelet count is first to rise. The increase in other bone-marrow elements takes place in the following order: hemoglobin, red cells and finally leucocytes.

NORMAL STANDARDS.

When a test is first discovered, the standards set for normal are usually correct but the normal variation is too narrow. Each individual has his or her normal standard and physiological variation. These standards are remarkably similar in different persons. When many are examined however, figures far from the average are encountered in a few healthy individuals and so the normal limits are widened. If, in any examination, a healthy individual has a reading at the top limits of normal and by later undergoing some experience or illness, his rating is changed to the lower limit of normal, he may suffer distress through this change although our subsequent examination by itself shows nothing unusual. Similarly a change from the lower to the top limits of normal may cause illness. Even when within normal limits, a result should be considered in relation to the average normal.

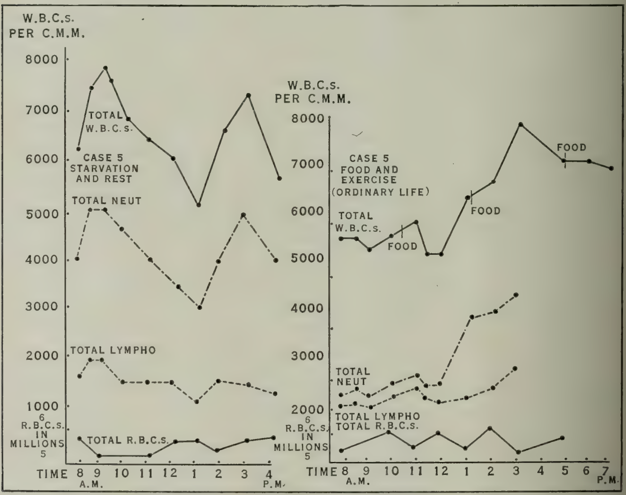

FIG. 27.—Hourly variation in cell counts during the day. A similar night tide starts in the evening, attains its height in the hours after midnight and ebbs away in the early forenoon, The curve of the neutrophile leucocyte is largely responsible for the variation in the total white cell counts. Practically the whole range of variation in the differential leucocyte count in a large series of healthy persons of the same age group can be traversed by a single individual in twenty-four hours. The total number of leucocytes is influenced by the personal factor. There is a uniform distribution of leucocytes in the peripheral and central circulations. (Shaw: Jour. Path. and Bact. 1927 xxx, 1.)

Although the unit or quantitative system in diagnostic procedure is valuable in giving definite data, other information is frequently necessary to arrive at a correct conclusion. Results obtained from previous examination, while the patient was free from symptoms, are valuable.

This problem is present with most quantitative estimations but a concrete illustration may be supplied from red blood counts. The first normals were given as 5 million red cells for men and 4.5 million for women. The latest figures show that the highest normal limit is 6.4 million and the lowest 4.1 million. I have obtained repeated blood counts around 7 million on a healthy medical student over a period of two years. Even after giving three transfusions his blood count remained around 7 million and a most complete history and physical examination revealed no abnormality. Up to the present, five years after the first blood count, he has worked hard as a student and intern and later as practitioner and has remained in perfect health. It is more than probable that this individual would complain of all the symptoms of anemia if some circumstance or illness would bring his blood count down to 4.5 million.

I have seen patients suffering from polycythemia vera with red blood counts around the upper limit of normal, one at 6.5 million, the other at 7 million. Both had all the clinical signs of polycythemia including plethoric lividity, asthenia, nasal hemorrhages and splenomegaly. The heart was normal and on exhaustive examination no cause could be found for the polycythemia. These patients were entirely free from symptoms after bleeding and phenylhydrozine which reduced the blood count to less than 5 million. Their symptoms returned in less than a year when treatment was discontinued and the blood count mounted to around 7 million. The normal and pathological limits appear to overlap. In a patient who has a blood count slightly above 4 million one frequently sees symptoms disappear when the count rises to over 5 million but it is difficult to be certain that the recovery was due to the improvement in the blood count.

In such problem cases an estimation of the following data would be valuable if time would permit.

Hemoglobin, grams per 100 cc.

Red cell count per cubic millimeter in millions.

Total red blood cells in the circulation in trillions.

(Total red blood cells in trillions equals red blood cells per cubic millimeter multiplied by total blood volume in cubic millimeters.)

Total blood volume in liters.

Total cell volume in liters.

Further than this we would like to measure accurately the relative size of the heart and great bloodvessels and to know whether the patient is of the robust, athletic type doing much work and requiring a well-developed vascular system and much blood or whether he is of a plump hypotonic type who has a diminutive vascular system and engages in only a minimum of physical activity.

CHAPTER II.

BLOOD CHEMICAL TESTS.

HEMOGLOBIN DETERMINATION.

Hemoglobin Determination Using the Newcomer Standard.—This method is the most accurate of the rapid clinical methods but it requires a special cup and plunger colorimeter which limits its use to fully equipped laboratories. The method consists in treating blood with hydrochloric acid to form the brown acid hematin. This is placed in one of the colorimeter cups and compared with the brown glass standard in the other. This glass standard is very carefully made of a definite thickness and shade of color so as to give a definite shade of brownish-yellow. Its main advantage is its permanency. Most are very accurately made although a few have been reported as showing a 10 per cent error. They should be controlled by noting the hemoglobin estimation on patients having exactly 5 million red cells per cubic millimeter or when possible checking them against Van Slyke oxygen-capacity method.

Technique.—Keep on hand a number of small labelled bottles containing exactly 5 cc. of tenth-normal or 1 per cent hydrochloric acid. To 5 cc. of the tenth-normal hydrochloric acid add *exactly* 20 cu.mm. of freshly drawn blood and mark the bottle with the person's name if a number are being done at once. Mix well and place this acid hematin fluid in one cup of the colorimeter. The other cup should contain the glass standard and some water. Match the color by taking two readings, determine the average of the colorimetric readings and obtain the grams of hemoglobin per 100 cc. of blood by dividing the colorimetric reading into the factor figure for the time since dilution and the thickness of the colored glass in millimeters.

Newcomer's Table for Use With the Standard Disk.

Thickness of colored glass in mm.		0.95	0.96	0.97	0.98	0.99	1.00	1.01	1.02	1.03	1.04	1.05
Minutes after dilution	10	94.4	95.4	96.4	97.4	98.4	99.4	100.4	101.4	102.4	103.4	104.4
	15	93.1	94.1	95.0	96.0	97.0	98.0	99.0	100.0	101.0	102.0	103.0
	20	92.5	93.5	94.5	95.4	96.4	97.4	98.4	99.4	100.4	101.3	102.3
	30	91.8	92.8	93.8	94.8	95.7	96.7	97.7	98.6	99.6	100.5	101.5
	40	91.6	92.5	93.5	94.5	95.4	96.4	97.4	98.3	99.3	100.3	101.2
Final		90.6	91.6	92.5	93.5	94.4	95.4	96.4	97.3	98.3	99.2	100.2

To obtain grams hemoglobin per 100 cc. blood divide the colorimetric reading into the appropriate figure.

Example.—With a colorimetric reading of 8.8 obtained fifteen minutes after dilution and compared with the colored glass 1.04 mm. in thickness, divide 8.8 into 1.02. The result, 11.6 is the grams of hemoglobin per 100 cc. of whole blood. Where the hemoglobin is likely to be under 50 it is best to use a double quantity of blood in the 5 cc. of tenth-normal hydrochloric acid. For the equivalent percentage of hemoglobin, see tables page 126. For the cell hemoglobin content see the estimation of the saturation index on page 72.

Hemoglobin Estimation by the Paper Scale (Tallqvist).—After cleaning the skin and needle, prick the ear with a quick stroke so as to make the blood flow without much pressure. Touch one of the small detached sheets of absorbent paper to the drop, fold the paper and blot the drop at once. This gives a layer of blood having a uniform thickness. Compare the color immediately with the scale at the back of the book using daylight coming from over the shoulder. Normal 80 to 100 per cent.

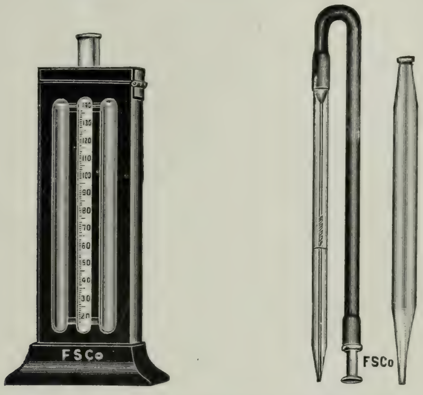

FIG. 28.—Sahli hemoglobinometer with non-fade standard.

Margin of error 10 to 20 per cent depending on the experience and skill of the examiner. No physician should be satisfied with such a possible error as this. The hemoglobin figures and color index estimations based on them may be very misleading. It is not possible to detect any hyperhemoglobinemia by the paper scale. The paper scale method should be replaced by the Sahli acid hematin method now that Sahli instruments may be obtained with permanent standards at a low cost and are accurate to within 5 per cent if the apparatus be standardized. It requires less than ten minutes to do a Sahli acid hematin estimation.

Hemoglobin by the Acid Hematin Method. (Sahli.)

Apparatus.—Sahli hemoglobinometer.[1]

A pipet to measure 0.20 cu.mm. of blood and one to convey acid and water to dilution tube.

One ounce of decinormal hydrochloric acid.

Blood lancet, absorbent cotton and alcohol.

THE ESTIMATION IN BRIEF.—Place 20 cu.mm. of blood from the pipet into the graduated tube which contains tenth-normal hydrochloric acid up to the "10" mark, and shake to mix. Dilute with tenth-normal hydrochloric acid until the solution matches the standard. The figure opposite the top of the column of solution denotes the percentage of hemoglobin.

The Estimation in Detail.—First place tenth-normal hydrochloric acid into the diluting tube up to the "10" or "20" mark, by means of the larger pipet. The lobe of the ear or the dorsum of the finger between the nail and first joint are the optimum places to obtain blood. Make a puncture not less than $\frac{1}{8}$ inch so that the blood will readily ooze out. Wipe away the first drop and after a large one has again appeared fill the pipet until the blood comes to the 20 cu.mm. mark. Wipe the blood off the tip and deposit the contents of the pipet in the graduated tube which contains the tenth-normal hydrochloric acid, rinsing it to wash all the blood into the graduated tube. Shake the graduated tube to mix the acid with the blood before it clots. Wash the 20 cu.mm. tube with water and dry it with acetone, in the same manner as a leucocyte pipet. Also clean off the lancet. Although the directions usually call for dilution with water, tenth-normal hydrochloric acid is better because it develops the full acid hematin color more readily, as the color depends on the amount of hemoglobin present, the larger amount of acid does not make for error. With tenth-normal hydrochloric acid in the larger pipet, add a few drops at a time to the brown acid hematin in the graduated tube, placing the finger over the end of the tube and invert to mix the contents between each amount added. As the color approaches the standard, add just a drop at a time, mixing the fluids by inverting between each drop. When the color matches the standard, note the figure opposite the top of the column in the dilution tube. Some makes have two series of graduations on the tube, a white one, the Sahli in which 17.3 gm. equals 100 per cent; the red figures in which 15.6 gm. equals 100 per cent register higher and should be followed.

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[1] Use only the type with tinted solid glass, non-fade standard. Formerly the standard was a tube containing colored liquid and if exposed to the light over a period of months it faded.

To Standardize the Hemoglobinometer.—Some of the graduated dilution tubes vary slightly in size and so give incorrect readings. For these the correction may be made by carefully estimating the hemoglobin of three or four normal persons whose red cell counts are from 4.9 to 5.1 million. Their hemoglobin should be between 95 and 105. If they are below 95 or above 105 the average hemoglobin reading at which these normals match the standard may be taken as the true 100 per cent. Suppose it be 85 per cent on the tube, then 15 per cent should be added to every reading taken. Thus if a reading is 65 on the tube, the true value is 65 plus 15 per cent of 65, or 75 per cent. The standards being of colored glass, if an apparatus is once checked and found correct, it will remain so indefinitely.

This is a very practical and accurate method of checking up a hemoglobinometer. One of the chief values of a correct hemoglobin reading is the determination of the color index and the above method insures the accuracy of this relation.

Another means of checking the accuracy of a hemoglobinometer, is to make an estimate with it on the same drop of blood that is being estimated by the use of the Newcomer colored disk standard in a colorimeter. The difference should be less than 5 per cent.

Interpretation.—Briefly stated the normal hemoglobin is 85 to 110 for adult males and 10 per cent lower for adult females. At birth it varies from 110 to 150 but falls so that at one year it is 65 to 85 per cent and makes a gradual ascent to reach the normal figures at sixteen years of age.

The chart represents quite well the normal range indicating the variations for age. Under basal conditions there is very little hourly or daily variation so that when possible it is better to make the estimation in the morning before the patient has had breakfast. With normal activities the variations during the day are less than 10 per cent.

Subnormal Hemoglobin, Anemia.—A diminution in hemoglobin is the earliest positive sign of anemia. The exceptions to this rule are very few indeed. In adults over 65 per cent hemoglobin is a mild anemia, 50 per cent to 65 per cent is a moderate anemia and below 50 per cent should be regarded as severe. In children three months to three years of age these figures should be lowered 10 points. Patients usually complain of weakness, breathlessness and palpitation when the hemoglobin approaches the 50 per cent level.

If the hemoglobin is at the lower limits of normal or when the clinical evidence favors anemia, a blood smear should be examined for irregularity in size and shape of the cells. If the smear appears normal it is not necessary to make further blood examination but if the hemoglobin be low or the cells irregular in size a complete blood cell examination including red blood cell count with the percentage of reticulocytes, a platelet count, a total and differential white cell count should be undertaken. When this is completed one should investigate the cause of the anemia systematically as outlined under anemia (page 95).

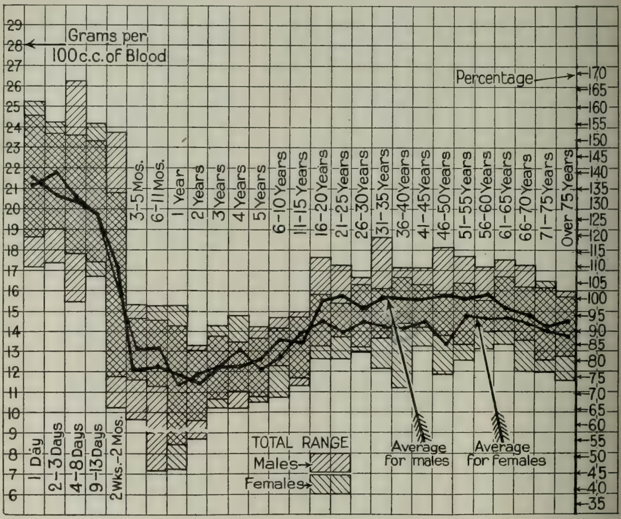

FIG. 29.—Chart of normal hemoglobin variation with age and sex. This chart is based on 919 estimations made by Williamson in Chicago on normal subjects of varying ages. To obtain the maximum in health, the difference between the grand average and the average of all readings above normal is doubled. The minimum is obtained in the same manner. All figures are lowered 8 per cent so that 15.6 gm. of hemoglobin equals 100 per cent as determined by Haden who found the average hemoglobin to be 15.6 on healthy individuals having red cell counts of 5 million. This brings the values in line with the recent findings of Osgood, Gram and Norgaard. In 16 healthy children six months to two years of age, I have found none below 9.5 gm. Values below that are probably subnormal.

Increased Hemoglobin.—In infants an increased hemoglobin content of the blood is normal. It is normal in adults at high altitudes. In disease hyperhemoglobinemia occurs where there is any embarrassment to the circulation in the heart such as mitral stenosis or congenital narrowing of the pulmonary artery. It also occurs in many cases of advanced emphysema and asthma. It is present with the high red cell count in erythema or polycythemia vera.

The hemoglobin estimation is of very definite use in the detection of dried out states especially in enteritis, vomiting and high fevers. Here the hemoglobin increase is a good index to the degree of anhydremia, lessened blood volume and increased viscosity which work a great hardship for the heart in these conditions. The hemoglobin estimation is a good control to determine the amount of fluid required by a patient in a dried out state. It is a more accurate index than the feel of the skin, the dryness of the tongue and the appearance of the soft tissues around the eyes.

COLOR INDEX.

This is the index most frequently used in the diagnosis of blood disease.

$$\text{Color index is } \frac{\text{Hemoglobin percentage.}}{\text{Red cell count percentage of 5 million.}}$$

It can be most readily estimated by dividing twice the first two figures of the red cell count into the hemoglobin percentage. A very low hemoglobin with the red cell count not decreased in the same proportion gives a color index less than 1 and indicates a secondary or chlorotic anemia. When the hemoglobin is not decreased to the same extent as the red cell count the color index is greater than one and a primary pernicious anemia may be suspected. Intestinal parasites may give a similar picture and any eosinophilia of the blood should be noted and the stool examined for ova. One should also keep in mind the possibility of *x*-ray or benzol poisoning which may produce an aplastic anemia not unlike some types of Addison's anemia.

THE ICTERUS INDEX; A QUANTITATIVE ESTIMATION OF THE COLOR OF THE BLOOD SERUM.

PRINCIPLE OF THE TEST.—Blood is allowed to clot and the clear serum compared with standards to record the depth of its color which depends on bile pigments.

Precautions.—1. *Hemolysis.*—All apparatus coming in contact with the blood should be clean and dry. Water or chemicals will cause hemolysis of the cells and readings cannot be made.

2. *Cloudy Serum.*—During digestion and food absorption the serum is cloudy and this greatly interferes with the test. The blood should be taken before breakfast or five hours after a meal, when the serum is clear. The estimation should be made within twenty-four hours.

Macroscopic Method Using Venous Blood.—*Apparatus.*—Colorimeter with Killian glass disk icterus index standards or 1 in 10,000 potassium dichromate, which should be kept in a dark place and prepared monthly from a 1 per cent solution. Blood vacuum tube, 5-cc. syringe and 22-gauge needle, 2 test-tubes.

The Test.—Obtain 5 cc. of blood from a vein in the arm. Let it stand in a clean dry test-tube until the clot contracts. An hour or two at body temperature will be sufficient but a longer time is required at room temperature. Centrifuge at a high speed for five minutes to pack the clot in the bottom of the tube. Without disturbing the clot pipet off 2 or 3 cc. of clear serum. A hypodermic syringe with a long needle is useful for this. Compare the serum with a 1 in 10,000 potassium dichromate solution which represents an icterus index of 1.

(*a*) For those having a standard colorimeter it is best to procure a Killian glass disk icterus index standard and after matching the color divide the thickness in millimeter of the serum in the other cup into 15. If this glass disk is not at hand a 1 in 10,000 potassium dichromate solution may be used as a standard and the cup set at 15 mm. depth. Move the other cup containing the serum until the shades match. The depth of the cup containing the unknown in millimeters divided into 15 is the icterus index.

Example.—Suppose the unknown cup when it matches has a depth of 3 mm., the icterus index would be 3 into 15 or 5. If the icterus index is over 15 dilute the serum 1 in 10 with normal saline and multiply the result by 10.

(*b*) For those not having a colorimeter, results satisfactory for clinical purposes may be obtained by test-tubes. Use two test-tubes about $\frac{3}{4}$ by 6 inches. They must be of equal size. Into one place about 10 cc. of 1 in 10,000 potassium dichromate standard solution and into the other place exactly 2 cc. of the clear serum. Face the light and hold a sheet of thin paper behind the tubes. To the serum add measured amounts from a graduated pipet of 0.9 sodium chloride solution until the color is similar to the one of the standard. The icterus index is the cubic centimeter of serum originally in the tube divided into the total of serum and saline required to make it the same color as the standard.

Example.—If to the 2 cc. of serum 13 cc. of saline have to be added to make the color similar to the standard 2 divided into 2 plus 13 equals 7.5 which is the icterus index. If only 1 cc. of serum is available the calculation would be 1 divided into 1 plus 7 (cubic centimeters of saline added) equals 8 with is the icterus index. If the icterus index is very high it is best to make a preliminary dilution of 1 in 10, using normal saline as diluent and multiply the result obtained by 10.

(*c*) For those making frequent clinical estimations the LaMotte-Pigford Icterus index comparator is useful and inexpensive.

The blood serum is obtained in the usual way, placed in a test-tube of equal diameter to standards and a comparison made behind a ground-glass window in the cover of the apparatus. It is necessary to have the serum entirely free from turbidity. If the icterus index is very high the serum may be diluted 2 or 3 times with saline. The tube that contains the serum has graduations for dilution purposes.

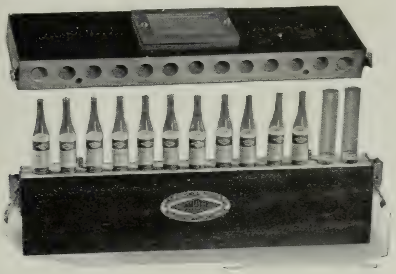

FIG. 30.—LaMotte-Pigford icterus index comparator.

PRACTICAL INTERPRETATION OF THE ICTERUS INDEX.

1. Normal 4 to 6.
2. Latent icterus 6 to 18.
3. Bile in the urine and jaundice at 18 and up.
4. Secondary anemia 2 to 4.

Hemolytic States:

5. Pernicious anemia 6 to 20.
6. Pneumonia and septic infections 4 to 15. (Above 10 in fatal cases.)
7. Chronic decompensated cardiovascular disease 4 to 15. (Above 6 in passive congestion of the liver.)

Disease of Biliary System:

8. Cholelithiasis 4 to 6.
9. Cholecystitis 7 to 15.
10. Occlusion and cholangitis of the common bile duct 30 to 150.

Other Diseases:

Gastric ulcer rheumatic fever, syphilis, tuberculosis 4 to 6. Duodenal ulcer 7 to 14. Diabetes 7 to 15. Carotinemia (from eating carrots) 6 to 15.

Interpretation of Icterus Index.—Slight jaundice is difficult to recognize in dark-skinned patients and the icterus index is very valuable in its detection. When the icterus index rises to 18, bile appears in the urine and there is a staining of the sclerotics and other tissues causing jaundice. Between 6 and 18 there is a latent jaundice with no bile in the urine and very little staining of the tissues. This occurs in hemolytic infections and pernicious anemia. The icterus index is high in the crisis of pernicious anemia and it becomes normal in the remission before clinical improvement is evident. If a marked hemorrhage occurs the icterus index may become sub-normal. Fifteen per cent of the patients with pernicious anemia and 15 per cent of the patients with secondary anemia have normal icterus indexes. In secondary anemia, which has a low icterus index, a pneumonia, a cardiac insufficiency with passive congestion of the liver or slight cholecystitis may cause a high reading. A high icterus index occurs in patients receiving too much arsphenamine and the test repeated at regular intervals is a good indicator of the hepatic tolerance for this drug. The bile absorption through a duodenal ulcer increases the icterus index and in diabetes there is a blood pigment resembling bile. One should always keep in mind the possibility of carotinemia in patients especially children, on a diet, consisting of much vegetable. Carrots contain the highest percentage of the pigment but oranges, squash, pumpkins, spinach, greens of all sorts and egg yolk contain amounts sufficient to color the blood serum *if such diets are taken in large amounts.* This is probably the cause of the high readings usually found in diabetes. The state may be excluded by the history of excess of the above-mentioned vegetables in the diet and a diffuse yellow pigmentation noticeable only on the palms of the hands and soles of the feet in contrast to the true icterus which affects the sclerotics and general cutaneous tissues in obstructive jaundice.

A patient with gastric carcinoma may have a low icterus index from the anemia and it may later go up when the secondary growths invade the bile ducts or liver. A constantly high reading points to new growths. Where biliary obstruction is temporary as from a calculus the icterus index becomes normal in about twenty-four hours after the obstruction is overcome, whereas the jaundice may remain for several weeks. Thus the icterus index indicates the true state of affairs much better than does the jaundice.

Disease of the lower biliary passages may be further investigated. Bile recovered by the duodenal tube may show microscopically clumps of bile-stained pus, columnar epithelium, crystals of cholesterol and calcium bilirubin. The pus and epithelium point definitely to empyema of the gall-bladder or infection of the bile ducts; the crystals to potential stone formation. Gall-bladder function may be investigated by x-ray examination for gall-bladder visualization following the ingestion of tetra-iodophthalein. Total absence of bile in the stool as determined by chemical tests is strongly suggestive of common bile duct occlusion due to new growth. Small amounts of bile may be present in clay-colored stools. Bile will be present in the urine where there is obstruction of the lower biliary passages or hepatitis producing an icterus index above 18. Bile is never present in the urine of a patient having hemolytic jaundice.

THE BROMSULPHTHALEIN TEST OF LIVER FUNCTION.

Outline of Technique (Rosenthal).—Inject intravenously an amount of the solution containing 2 milligrams of the dye for each kilogram ($2\frac{1}{5}$ pounds) of body weight. Recently 5 mgm. has been given and no dye remains normally in forty-five minutes. In thirty minutes withdraw 10 cc. of blood from a vein on the other arm. When a clot has formed, centrifuge and place 2 cc. of the clear serum in each of two narrow test-tubes of the same diameter as those of the colorimeter. To one of the tubes add 2 drops of 5 per cent solution of sodium hydroxide and to the other a similar volume of 5 per cent hydrochloric acid. Compare with the standards by placing the acidified serum behind the standard and a tube containing water behind the alkalinized serum. (The 100 per cent standard is calculated on the blood volume and would represent the amount of dye in the serum if none were removed.)

To prepare the standards add 4 mgm. of the dye to 100 cc. of water and add 4 drops of 5 per cent sodium hydroxide. This gives the 100 per cent standard. Ten standards ranging from 10 to 100 per cent may be prepared from this by diluting with alkaline water. They will keep for six months in the dark. Prepared standards may be purchased from Hynson, Westcott & Dunning, Baltimore, Md.

Interpretation.—The bromsulphthalein test measures liver function quantitatively. Normal persons excrete practically all the dye in half an hour. There is never more than 5 per cent remaining in the blood. In advanced liver disease 100 per cent of the dye may remain in the blood stream and where there is only 50 per cent function of the usual liver requirement, there will be a dye retention of 50 per cent.

This test is useful in detecting hepatitis, pre-eclamptic toxemia, advanced cirrhosis, Banti's disease, leukemia, malignant metastases of cancer and obstruction of the biliary passages. Although one of the best tests of liver function, it does not differentiate the various liver diseases.

THE VAN DEN BERGH REACTION.

Principle.—Clear serum is treated with Ehrlich's diazo reagent and if bilirubin be present a red color results. Alcoholic extraction of the bilirubin from the blood serum is necessary for the indirect reaction.

The Test.—Obtain 1 cc. of serum as outlined for the icterus index test. Add 2 cc. of water and 0.5 cc. of the diazo reagent. (Solution A.—Sulphanilic acid, 1 gm.; hydrochloric acid, concentrated, 15 cc.; distilled water, 1 liter. Solution B.—Sodium nitrite, 0.5 gm.; distilled water, 100 cc. Immediately before the test mix 25 cc. of Solution A with 0.75 cc. of Solution B and use this as the diazo reagent.)

The Reaction.—(1) Immediate, a reddish-violet color within one minute. (2) Delayed reaction—beginning after one minute and reaching a maximum by ten minutes. (3) Biphasic reaction—a faint color in about a minute which continues to deepen for five minutes. It is mid-way between the immediate and delayed reactions.

Indirect Reaction.—To 1 cc. of serum add 2 cc. of absolute alcohol. Shake and centrifuge for five minutes. Pipet off 1 cc. of the clear supernatant fluid. Add 0.5 cc. of alcohol and 0.25 cc. of the diazo reagent. This is compared with a standard (cobalt sulphate crystals containing water of crystalization 3.92 gm. in distilled water 100 cc.). The bilirubin in the blood serum has been diluted five times. Therefore in obtaining the units of bilirubin the result must be multiplied by 5. A unit of bilirubin is 0.5 mgm. per 100 cc. of serum. Normal 0.1 to 0.25 mgm. per 100 cc.

Interpretation.—The direct reaction points to obstructive jaundice while the delayed or indirect points to a hemolytic or toxic form. The biphasic frequently occurs in liver infections formerly called catarrhal jaundice and now known to be acute hepatitis.

Broadly speaking mild jaundice where the pigment accumulates slowly and is bound with the blood plasma give only indirect reactions. This occurs in the hemolytic states and where jaundice has been of long-standing. Obstructive jaundice of recent origin with free solution of bile in the blood that has not joined up with the plasma gives direct reactions. The test is not a satisfactory one but is outlined here because of the frequent reference to it in recent medical writings.

Testing the urine for bile is a more expedient examination. Bile is present in obstructive jaundice or hepatitis and an excess of urobilin will be found. Bile is never present in hemolytic jaundice; urobilin is never present in complete biliary obstruction such as results from new growth.

The Origin of Bile Pigments.—The bile pigments come from the hemoglobin of disintegrated red cells. In health these broken down red cells are taken up by special endothelial cells which line the bloodvessels and form the reticulo-endothelial system. Most of these special endothelial cells occur in the bloodvessels of the liver, spleen and bone-marrow. In the liver the bile pigments are separated from the blood plasma and with bile salts go down the biliary ducts as pure bile. The bacteria in the intestine act on part of the bile to form urobilinogen and urobilin, which are absorbed through the intestinal wall into the portal circulation where they are mostly excreted through the liver into the bile ducts again.

A knowledge of this process is useful in diagnosis. Bile pigments bound to plasma cannot be excreted through the kidney. Bile separated by the liver from the blood plasma if dammed back by calculus into the biliary ducts and reabsorbed into the blood stream is readily filtered out by the kidney. That is, extra hepatic or hemolytic jaundice gives no bile in the urine and obstructive jaundice due to calculi in the common duct does give bile in the urine. If no bile enters the intestine as is frequently the case when a new growth involves the common bile duct, no urobilin will be formed so that the urine contains bile without urobilin. Any liver damage in which even small amounts of bile enters the intestine will give a very large urobilin and urobilinogen output in the urine. Practically all that is absorbed by the intestine is excreted by the kidney because the impaired liver cannot excrete it. Hemolytic or extra hepatic jaundice usually produces an icterus index between normal and 25. This causes an increased bile output and also an increased urobilin absorption, some of which is excreted by the kidney. The high icterus indices are usually due to obstruction of the biliary ducts, the slightly increased one to hemolytic states or slight liver impairment.

Liver Function Tests.—One may study any of the different functions such as the biliary, by its amount in the blood or in the excretions, the power to store levulose or galactose, by tolerance tests of these sugars, the urea nitrogen making up the total nitrogen in the urine, the fibrin forming capacity by an estimation of the fibrin content of the blood, the antitoxic power by the presence or absence of glycuronic acid in the urine following 5 grains of aspirin or the power of the liver to excrete a dye such as bromsulphthalein. Any of these functions may be more deranged than another in chronic disease. Only in diffuse acute disease are all the functions impaired simultaneously. The information gained by estimation of several of the functions does not justify the time required to do them or the annoyance to the patient.

The liver suffers from a variety of diseases, passive congestion, fatty degeneration, acute and chronic inflammation, mechanical obstruction of the bile outlet and malignant tumors. Function may be impaired in any of these diseases and mechanical block by a calculus may totally recover while malignant new growths are fatal. Liver function tests do not indicate the specific disease causing the disorder.

The icterus index is chosen as a liver-function test because it is important, easily performed and well established. It is essentially a test of biliary function and shows only a slight variation in cirrhosis because the impaired liver has adapted itself to its biliary task. In practically all other diseases however it is one of the earliest to show change.

Effect of Bile Retention.—Acute cholecystitis is usually accompanied by acute infection ascending into the biliary tracts throughout the liver and producing a slight biliary obstruction and an acute hepatitis. If a stone or new growth produces a prolonged obstruction to the bile outlet the liver will be markedly damaged. At first there is enormous dilatation of the tiny bile capillaries in the liver producing an edema and swelling of the whole organ. The icterus index rises within several hours of the onset, bile appears in the urine in one hour and jaundice is usually present the following day. This state is known as hydrohepatosis.

If the obstruction persists a marked degeneration of the liver cells soon occurs and much of the former liver substance is replaced by a cirrhosis in less than a month.

An Aid in Understanding Liver Disease.—We should think of liver disease as acute, subacute and chronic. Kidney disease in its various stages is much better known than liver disease and because there is a marked similarity between the two a comparison may help us to understand liver disease. Acute hepatitis resembles acute nephritis. Both are usually diffuse and if not too severe recovery takes place.. Both organs have considerable regenerative power. Acute diseases are usually due to toxins in acute infections but many of the acute liver diseases result from gastro-intestinal absorption of toxins which reach the liver before entering the general circulation and produce various histological states such as cloudy swelling, fatty degeneration and in the very severe cases such as acute yellow atrophy, complete necrosis of the parenchymatous hepatic cells.

Septic disease is very similar in the liver and kidney. In the liver we have solitary or multiple abscesses, more frequently due to amœba than bacteria and they correspond to the septic pyemic kidney with localized abscess. In both organs the function is good if enough normal organ be left to carry on the required function.

Chronic interstitial nephritis is more commonly the aftermath of several acute or subacute attacks than it is the outcome of a chronic progressive sclerosis; and so the liver cirrhosis is very often the outcome of numerous attacks of acute or subacute hepatitis as well as a slow progressive change. In these organs the reserve is several times greater than the actual requirements and it is only after this reserve is destroyed by the disease that the functional tests begin to show changes. These tests are therefore not early indicators of disease. Disease of the lower urinary tract with its ureters, urinary bladder and urethra has many points in common with disease of the lower biliary tract with its hepatic ducts, its gall bladder and its common ducts. Calculi are the results of a disturbed mineral metabolism and, in the kidney at least, infection plays a part. Because secondary growths from the stomach and bowel frequently invade the liver it is more subject to tumors than the kidney but growths pressing on the bile ducts, especially in the head of the pancreas, produce much the same effect on the liver as prostatic enlargement does on the kidney. There are many definite differences between these two systems such as their function and relation to the gastro-intestinal tract but the likeness in the gross structure and reaction to disease is striking.

Interpretation in Detail of the Biliary Function in Jaundice.—An increased icterus index may mean obstruction disease anywhere along the bile ducts, hepatic insufficiency or an excessive production of bilirubin through increased red cell destruction. The highest figures come from obstruction of the bile ducts. Obstructive new growths give constantly high figures up to and over 100, while obstructive calculi and catarrhal states give figures from 25 to over 100 which fluctuate very much. This fluctuation is not apparent from the appearance of the skin. There is bile in the urine but little or none in the stool. The hemolytic states, such as septic infections and pernicious anemia, give figures from 6 to 25 and there is bile in the stool but not in the urine although its urobilin content is increased. The origin of bilirubin is not definitely settled. For many years the liver has been regarded as the sole site of bile production. Recently attention has been focussed on the extrahepatic origin of bilirubin. In hemolytic icterus the spleen appeared to be the chief site of red cell destruction and bilirubin production as its removal cures the disease. Experimental work by Mann at the Mayo Clinic shows that all but a small amount of bile comes from the bone-marrow. Of course in these experiments it is just possible that the endothelium of the bone-marrow has taken on the production of bilirubin from degenerated red cells, when the spleen and liver are removed in somewhat the same manner as the excretory organs and skin take on part of the kidney function when the kidneys are removed. This change may be brought about by almost any cells in the body as demonstrated by the color changes of blue, green and yellow which occur in the skin following the subcutaneous injection of blood.

There is probably much truth in the belief that the liver is the main producer of bilirubin and bile in health. Were this not so that view would not have held sway for so long. The difficulty is in devising experiments that do not upset the normal functions so that before we divorce the liver from the rôle of bilirubin manufacturer we should have very conclusive evidence.

The Van den Bergh test has done much to popularize the idea of hematogenous jaundice. Van den Bergh himself and McNee have claimed that the indirect reaction occurs only in extrahepatic hematogenous jaundice because the bilirubin is firmly bound to the serum whereas the direct occurs in obstructive jaundice where the bilirubin has been separated from the serum by passage through the liver and is reabsorbed into the blood stream. They state that catarrhal jaundice where the liver cells are damaged a biphasic reaction occurs. This consists of a combined marked indirect reaction due to the bilirubin which has not been acted on by the liver cells and slight direct reaction due to the bile which has been separated from the blood serum and reabsorbed from the bile ducts into the blood stream.

This seems like making a test tell a great deal and these claims have been ably refuted by Blankenhorn who maintains that large amounts of bile thrown suddenly into the blood will give the direct reaction while small amounts which can be taken up by the blood plasma give the indirect reaction. Where jaundice has been present for a long time the bile is firmly bound to the plasma and gives only the indirect reaction although the total bile content may be high. Personal observation of a number of delayed direct reactions with marked indirect reactions have led to confusion rather than giving information.

When obstructive jaundice has been present for several weeks the entire biliary system in the liver spleen and reticulo-endothelial cells lining the bloodvessels is damaged and can only perform its function in a feeble manner. The bile is pale and is sometimes described as white bile. The icterus index may be around 25 and the van den Bergh, biphasic. There will be a high retention of bromsulphthalein. If the obstruction can be removed, the bile will, after a few days, start to regain its greenish regenerative powers of the bile-forming system.

BLOOD SUGAR ESTIMATION USING VENOUS BLOOD.

PRINCIPLE.—The protein is removed from the blood by tungstic acid and the protein-free filtrate is heated with copper tartrate solution. The dextrose and an excess of the copper tartrate form a yellowish oxide of copper, the amount of which depends on the amount of dextrose present. This copper oxide when mixed with molybdate

phosphate solution forms a bright blue color the intensity of which depends on the amount of oxide present; that is a deep blue color is obtained with a high blood sugar, a pale blue with low blood sugar.

By comparison of the color in a colorimeter with a standard obtained by treating 0.1 per cent of pure dextrose solution in the same manner as the blood has been treated, the percentage of sugar in the blood may be estimated.

Apparatus. For Occasional Use.—The vacuum tube either of the Keidel or Venule type is most convenient. They must contain oxalate to prevent clotting.[1]

For Frequent Use.—All glass syringe, 5 or 10 cc., with vaseline coated piston with a 22-gauge needle, or a 20-gauge needle and test-tube containing 1 drop of 20 per cent potassium oxalate for each 5 cc. of blood.

Tourniquet of rubber tubing, alcohol and absorbent cotton.

Obtaining the Specimen.—Obtain 5 cc. of blood from the most prominent vein at the volar surface of the elbow. The technique of vein puncture is fully described under the blood Wassermann (page 196). If a needle be too large it is painful, if too small the bloodflow is inadequate. It should be very sharp. The blood must come in contact and be well mixed with the oxalate within two minutes from the time it comes from the vein. Gentle shaking of the test-tube or bottle containing the oxalate and blood will do this. The blood for a single examination should be taken before breakfast or when this is not possible, not sooner than four hours after the patient has eaten.

Preservation.—After one-half hour at room temperature the sugar content of the blood will begin to diminish. Even in an ice-chest where the glycolysis is less than at room temperature there may be 7 mgm. loss in two hours and 20 mgm. in four hours.[2] When analysis cannot be done within one-half hour after blood is withdrawn a preservative is necessary. The most readily available preservative is commercial formalin. One drop added to 5 cc. of blood, will prevent sugar-loss for twenty-four hours at room temperature but sometimes it gives an increased reading.

The best preservative is sodium fluoride. Ten to 20 mgm. per cc. of blood will preserve the sugar content for ten days at room temperature. One hundred milligrams of the powder should be placed in the tube for 5 cc. of blood. Shake the tube immediately after the blood is withdrawn, otherwise it will clot. As sodium fluoride only dissolves to 4 per cent it cannot be used in solution as is potassium oxalate. Oxalate is unnecessary with sodium fluoride which is an anticoagulant as well as a preservative. Fluoride is a satisfactory preservative for the non-protein nitrogen, uric acid, creatinine and cholesterol content of the blood. It is necessary to use special precautions in urea preservation. (See page 146.)

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[1] If only vacuum tubes containing no anticoagulant be at hand remove the rubber tube and place it in 2 drops of a 20 per cent sodium or potassium oxalate solution as an anticoagulant. This will be drawn into the test-tube with the blood.

[2] Johns, H. J.: Glycolysis, Ann. Clin. Med., 1925, **3**, 667.

Apparatus.

Special Folin-Wu blood sugar tubes.
Two 2-cc. Ostwald pipets.
Two Erlenmeyer flasks, funnel, filter paper, water-bath.

A colorimeter. (The cup and plunger type is best but very close readings may be obtained even with test-tube colorimeters. For the use of test-tube colorimeters see page 394.)

Reagents.

Use only chemically pure reagents for this test.
10 per cent sodium tungstate 500 cc.
$\frac{2}{3}$ normal sulphuric acid 500 cc.
20 per cent potassium oxalate 25 cc. or 1 ounce.
Powdered sodium fluoride 30 gm. or 1 ounce.

Standard Stock Dextrose Solution.—A stock solution of 1 per cent highest purity dextrose accurately made up in 0.25 per cent benzoic acid solution keeps indefinitely. From this make-up the standard 0.01 per cent solution monthly by diluting 5 cc. of the stock solution to 500 cc. with 0.25 per cent benzoic acid. For occasional very high blood sugars 0.02 per cent is necessary and is made by diluting 5 cc. of the stock solution to 250 cc.

Alkaline Copper Solution.—Dissolve 40 gm. of pure anhydrous sodium carbonate in about 400 cc. of water and transfer to a liter flask. Add 7.5 gm. of tartaric acid and after it has dissolved add 4.5 gm. of crystallized copper sulphate. Mix and add distilled water to 1 liter. If a sediment forms in time, filter the solution. It remains good indefinitely.

Molybdate Phosphate Solution.—Place in liter beaker 35 gm. molybdic acid, 5 gm. sodium tungstate, 200 cc. 10 per cent sodium hydroxide and 200 cc. of water. Boil vigorously for one-half hour. Cool and dilute to about 350 cc. and add 125 cc. of concentrated (85 per cent) phosphoric acid. Dilute to 500 cc. Keeps indefinitely.

The Test.—1. Place 2 cc. of well-mixed blood in a small Erlenmeyer flask.

2. Add 14 cc. distilled water and mix.

3. Add 2 cc. of 10 per cent sodium tungstate. Mix well.

4. Add, shaking between each few drops, 2 cc. of two-thirds-normal sulphuric acid. Cork and shake vigorously. The mixture should be chocolate-brown. After five minutes filter. The filtrate should be clear.

5. In one special Folin sugar tube marked "S" for standard; place 2 cc. of the standard sugar solution containing 0.01 per cent glucose. In another special Folin sugar tube place 2 cc. of the protein-free filtrate.

6. To each tube add 2 cc. of the alkaline copper solution. Place each in boiling water for six minutes. Remove, allow to cool and to each add 2 cc. molybdate solution.. Mix.

7. Dilute with water to the 25-cc. mark and mix.

8. Compare in the colorimeter.

Calculation, using a cup and plunger colorimeter.

$$\frac{\textit{Reading of standard} \times 100}{\text{reading of unknown}} = \text{mgm. of glucose per 100 cc. of blood.}$$

Estimations made using a test-tube colorimeter (see page 394), have an error less than 10 mgm. per 100 cc. of blood.

Precautions.—An excess of oxalate may cause a reddish color instead of the desired chocolate-brown. For this add 10 per cent sulphuric acid, a drop at a time, shaking vigorously after each drop until the brown color appears. This will give a clear filtrate, whereas the reddish mixture gives a cloudy one. The addition of acid should not be necessary and is not a desirable procedure but it may save an important specimen.

Interpretation.—*Methods Used.*—Different methods give different readings. The readings here mentioned are obtained by the Folin-Wu method, which is the one most widely accepted. The range of the normal fasting blood sugar is 80 to 120 mgm. with an average of 100 mgm. per 100 cc. of blood. More recent modifications of the test by Folin and by Benedict give results that are from 25 to 30 per cent lower. These figures are probably nearer the actual blood sugar content but the methods involve somewhat delicate technique and are not as yet fully standardized. The older picramic acid methods of Benedict and Lewis and their modifications gave readings that were 20 per cent or more, higher than the Folin-Wu. The very high readings obtained in some cases of hypertension caused the picramic acid methods to fall into disuse.

The blood is a much better indicator of the patient's carbohydrate metabolism than the urine.

Relation to Food.—A single specimen taken before breakfast or not sooner than four hours after a meal is of infinitely more value than one taken shortly after the patient has eaten. The fasting normal by the Folin-Wu method should not go above 120 mgm. *Any glycosuria in a patient with a fasting blood sugar below* 110 *mgm. is probably benign and not diabetic.* (*Knud Faber.*) Up to 140 mgm. is occasionally obtained in focal infections, hypertension or chronic arthritis. In any endocrine disorder hyperactive states, such as hyperthyroidism or hyperpituitarism, give blood sugars up to 140 mgm. while hypo-active endocrine states like Addison's disease give blood sugars at the lower limits of normal, Mild diabetes gives a fasting blood sugar from 150 mgm. to 250 mgm., while the severer types give fasting blood sugar from 250 to 600 to 800 mgm.

The blood sugar rises reaching a maximum one-half to one hour after meals and usually going higher after the morning meal than after the noon or evening meal. An elevation of long duration is significant of diabetes whereas a quick rise and decline suggests hyperthyroidism as will be more fully demonstrated in the glucose tolerance curve.

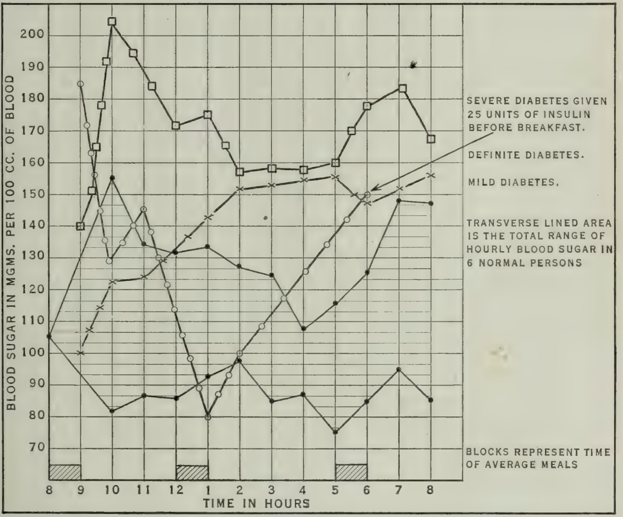

FIG. 31.—All-day blood sugar curves at hourly intervals. (Modified from Jonas, *et al.*, Arch. Int. Med., 1925, **35**, 289.)

Relation of Blood Sugar to Urine Sugar.—Sugar is usually excreted into the urine when it reaches a concentration of 160 to 180 mgm. per 100 cc. of blood. This point at which sugar appears in the urine is known as the renal threshold. In normal individuals the blood sugar rarely goes over 140 mgm. following an average meal. After a very large sugar intake the blood sugar may be elevated enough for several hours to produce sugar in the urine temporarily. This state is known as alimentary glycosuria. *In most diabetics the* *sugar percentage in the urine is proportional to the sugar percentage in the blood,* the mild cases with a blood sugar of 200 mgm. showing 2 per cent in the urine and the severe ones with a blood sugar of 400 mgm. showing 4 to 8 per cent glucose in the urine.

In patients who have had diabetes for a long time and have considerable arteriosclerosis and renal sclerosis, the blood sugar may be from 200 to 500 mgm. and *no* sugar be present in the urine. This is particularly true of the severe acute attacks with acidosis that occur in diabetes of long-standing following infection or dietary indiscretions.

In children the blood sugar may be normal in early diabetes and all the other typical symptoms, physical signs and *glycosuria* be present.

Renal benign glycosuria is characterized by a normal blood sugar and less than 1 per cent of sugar in the urine, which does not vary greatly with the amount of carbohydrate intake. There are none of the typical symptoms and signs of diabetes and the tendency is frequently hereditary. Most cases of renal glycosuria remain benign. A few, after a number of years, develop diabetes mellitus.

Hypoglycemia develops after an excessive dose of insulin. Values around the lower limits of normal are frequently encountered in poor nutrition. It also occurs in weak puny individuals where no definite cause can be found. It is wise to inquire about the time interval between removing the blood and its analysis. Glycolysis may have occurred!

THE SUGAR TOLERANCE TEST.

PRINCIPLE.—Where the sugar metabolism of the body is in question it may be tried out under a definite load by giving the patient glucose and then examining the blood and urine at hourly intervals. Two hours after 100 gm. of glucose the blood sugar of the average adult will return to normal and the urine will not show sugar at any time. This test is useful in detecting incipient or mild diabetes.

Technique.—The commonest method of doing the test is to take the fasting blood sugar, preferably in the morning, then to give 100 gm. of pure dextrose by mouth. It is obviously much better to vary the dose of dextrose with the weight of the body and 1.5 gm. of dextrose per kilo ($2\frac{1}{5}$ pounds) of body weight has been used and found satisfactory by Lennox.[1] Contrary perhaps to expectation a dose of 400 gm. of dextrose does not give a higher blood sugar than a dose of 50 gm. in a normal individual.[1] The curve however, forms a wider angle with the larger dose. Opinion differs regarding the frequency with which samples of blood should be taken. Taking specimens of blood every quarter or half hour over a period of three hours does not give enough extra information to justify the pain inflicted upon the patient and the labor of examining the many samples. One estimation on blood drawn before the glucose meal, at one and at two hours after the glucose meal will give the trend of the curve. Rarely a specimen taken three hours after the dextrose is of value but this reading is usually only slightly higher than the fasting blood sugar.

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[1] Lennox and Bellinger: Repeated Blood-sugar Curves in Non-diabetic Subjects, Jour. Clin. Invest., **4**, 331.

Sugar Tolerance Test.

Patient's Name..............................Date................

Blood Sugar (Folin-Wu)

Mgm. per 100 cc.	Fasting	1 hr.	2 hr.	3 hr.
400				
350				
300				
250				
200				
150				
100				
50				
0				

Urine Sugar, per cent

	Fasting	1 hr.	2 hr.	3 hr.
5				
4				
4				
3				
2				
1				
0				

Adult patients given 100 gm. of glucose.

Others 1.5 gm. per pound of body weight.

Examined by...................M.D.

Interpretation.—A fasting sugar over 120 mgm. per 100 cc. with glycosuria signifies diabetes and it is therefore unnecessary to proceed further with the dextrose tolerance test. Where there is a fasting blood sugar under 120 mgm. per 100 cc. and sugar in the urine occasionally an estimation of the glucose tolerance may be of value, especially in life insurance examinations. The diet for several days preceding the test influences it. This depends on whether there is an excess or restriction of protein, fat, carbohydrate or water. An average well-balanced diet should precede the dextrose tolerance test for several days.[1]

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[1] Hanson: Investigations in the Blood Sugar in Man, Acta med. scand., 1923, Supp. NP, **4**, 1.

The maximum normal blood sugar should not be above 180 *mgm. at one hour and should be down to* 140 *mgm. at two hours.* In diabetes the normal curve is sustained so that it is still over 150 mgm. in two or three hours. A steeple-shaped curve with fasting and two or three-hour specimens below 120 mgm. but the one-hour specimen over 200 mgm. points to hyperthyroidism or hyperpituitarism rather than diabetes. There may or may not be glucosuria. It is very important to distinguish these conditions. I have seen a man who had glycosuria and was suffering from severe exophthalmic goiter, given antidiabetic treatment whereas a later diagnosis revealed the true nature of the disease and marked improvement took place following thyroidectomy. This patient, a man, aged fifty-eight years, had a sugar tolerance curve of 120 mgm. fasting, 220 mgm. one hour after glucose and 90 mgm. two hours after glucose. His urine one hour after the dextrose contained 0.5 per cent. of sugar and two hours after 1.5 per cent. The physical examination and the basal metabolism test should differentiate these two states.

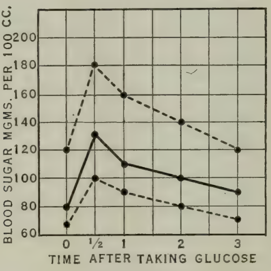

FIG. 32.—Normal dextrose tolerance curve and dotted lines indicating the upper and lower limits of normal curves.

When a low renal threshold can be demonstrated the glycosuria must be considered benign. When there is a rise to an abnormal height after the ingestion of sugar, more caution is necessary. The only type of curve from which definite diagnosis may be made is the diabetic one, which is abnormally high throughout. A positive diagnosis may be made from this even in the absence of clinical evidence.

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[1] Sweeney: Dietary Factors That Influence the Dextrose Tolerance Test, Arch. Int. Med., 1927, **40**, 818.

Abnormal types of sugar tolerance curves may occur in boils, carbuncles, eczema, sycosis, exophthalmic goiter, myxedema, Addison's disease, pituitary disorders and various nerve lesions.

ABNORMAL GLUCOSE TOLERANCE CURVES.[1]

1. *Abnormal Type of Curve:*	*Clinical Diagnosis.*
Normal fasting blood sugar 80–120 mgm. Normal peak one hour after glucose 120–180 mgm. *Return to Subnormal reading three hours after glucose* 60–90 *mgm.*	Addison's disease, lower motor neuron lesions. The fasting content is usually below 100 mgm.
2. *Abnormal Type of Curve:* Normal fasting blood sugar 80–120 mgm. Normal peak one hour after glucose 120–180 mgm. *Remaining high two to three hours after glucose* 120–180 mgm.	Mild diabetes, adiposity, skin sepsis, upper motor neuron lesion. This curve has no definite significance apart from demonstrating a slightly lessened sugar tolerance.
3. *Abnormal Type of Curve:* Normal fasting blood sugar 80–120 mgm. High peak one hour after glucose 180–300 mgm. Return to normal two to three hours after glucose 80–120 mgm.	Hyperthyroidism, pituitary disease, benign glycosuria, upper and lower motor neuron lesions, myasthenia gravis.
4. *Abnormal Type of Curve:* Normal fasting blood sugar 80–120 mgm. *High peak one hour after glucose* 180–300 mgm. *Slow return two to three hours after glucose* 140–240 *mgm.*	Mild diabetes, hyperthyroidism, hyperpituitarism, gastro-intestinal carcinoma and cirrhosis of the liver. Present in many diseases and therefore not of value in differential diagnosis although it shows a defective carbohydrate metabolism. A normal curve aids in ruling out the above diseases.

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[1] Modified from Hale, White and Payne: The Diagnostic Value of Dextrose Tolerance Curves, Quart. Jour. Med., 1927, **20**, 437. (Courtesy of the Oxford University Press.)

5. *Abnormal Type of Curve:*	*Clinical Diagnosis.*
High fasting blood sugar 140–300 *mgm.* High peak one hour after glucose 220–400 mgm. *Remaining high two to three hours after glucose* 200–350 *mgm.*	Pathognomonic of diabetes even in the absence of typical clinical signs and symptoms. The fasting sugar is high and the glucose curve therefore unnecessary.

The Hunt Micro-modification of the Folin-Wu Method.—The very small amounts of blood required gives this method an advantage over many other micromethods. Most of the results are exactly the same as those obtained by the standard method and with a fair degree of skill the variation is never greater than 20 mgm.

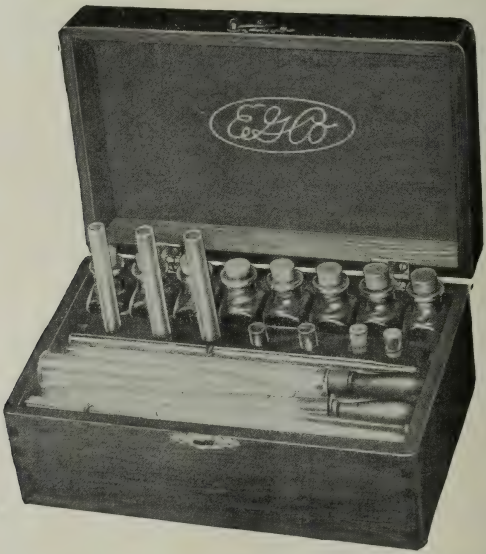

Fig. 33.—Micro blood-sugar apparatus. Hunt micro modification of the Folin-Wu method, used especially for children and coma cases requiring frequent blood sugar estimations. (Manufactured by the Emil Greiner Co., New York.)

Technique.—0.05 cc. of blood measured with a special pipet is put into a small conical tube containing 0.4 cc. of 10 per cent sodium tungstate. Then 0.05 cc. of two-thirds normal sulphuric acid is added and after shaking vigorously centrifuge for four minutes. Then place 0.1 cc. of the clear supernatant fluid into each of the two graduated tubes *B* and 0.1 cc. of the standard sugar solution into the standard tube *A*. To each of the three tubes add 0.1 cc. of the copper solution. Boil for six minutes. Cool and add 0.1 cc. of the phosphomolybdic acid reagent to each of the three tubes. Let stand one minute and add water to tube *A* until the line is reached. Mix well by inverting. This is the standard. Add water to the tube *B* to the first graduation. Hold the tubes side by side with a piece of white paper between them and the light and compare the colors. If the color in tube *B* be darker than that in tube *A*, add water to the next graduation at a time until the colors in tubes *A* and *B* match exactly. The figure at the level of the fluid in tube *B* represents the percentage of sugar in the blood. Use the second tube *B* to check the results obtained by the first.

Interpretation.—This method is useful for determining the blood sugar on a child or an obese patient where it may be difficult to find the vein, or on patients in coma where frequent estimations must be made. The capillary blood resembles the venous blood only when a patient is fasting. It gives unduly high readings in the glucose tolerance test because the capillary blood resembles the arterial blood which when glucose absorption is taking place has a higher sugar content than the venous blood.

BLOOD UREA NITROGEN ESTIMATION. STANDARD METHOD.

Principle.—Urease breaks down the blood urea into ammonium salts during the incubation. On adding potassium carbonate the ammonia is liberated and by aëration drawn through acid. The amount of urea present may be calculated by the amount of acid neutralized.

Reagents.

Urease tablets (Hynson, Westcott & Dunning or Squibb).
Antifoam solution made up of 20 per cent resin in turpentine.
Potassium carbonate powder 4 ounces.
$\frac{1}{50}$ normal hydrochloric acid or sulphuric acid and $\frac{1}{50}$ normal sodium hydroxide.
Indicator, 1 per cent alizarin.
Pipets, 5- and 10-cc.
3 test-tubes with rubber stoppers containing two holes in which are glass tubes to be connected with rubber tubing.
A suction pump attached to a water-tap.

The Test.—Crush 2 urease tablets in about 5 cc. of water and place in a large test-tube with exactly 3 cc. of whole blood. Add about 2 cc. of antifoam solution and incubate in hot water at 40° to 50° C. (the maximum heat the hand can bear) for one-half hour. Connect with the suction pump so that the air bubbles first through strong acid to neutralize any ammonia from the air, then through the blood and finally through the 10 cc. of $\frac{1}{50}$ normal acid to which has been added 2 drops of alizarin indicator. Now add 5 gm. (the amount that will stack on a dime) of potassium carbonate to the blood. Aërate for thirty minutes, at first slowly and later briskly. No contents of the blood tube should bubble up to the cork. Titrate the acid solution with $\frac{1}{50}$ normal sodium hydroxide. Multiply the number of cubic centimeters of acid used up, that is 10 cc. minus the amount of alkali titrated, by 9.34.

Example.—7.5 cc. of NaOH. turns the indicator from red to purple. 10 cc. − 7.5 = 2.5 cc. × 9.34 = 23 which is the milligrams of urea nitrogen per 100 cc. of blood.

Precautions.—10 cc. of the $\frac{1}{50}$ normal NaOH should exactly neutralize 10cc. of the $\frac{1}{50}$ normal acid. The air should bubble through strong acid before entering the blood tube and then it should bubble through a column of $\frac{1}{50}$ normal acid at least 2 inches high. A determination made occasionally on 3 cc. of distilled water instead of blood will reveal any source of error from ammonia in the chemicals and an estimation made on 3 cc. of accurately prepared 1 per cent chemically pure urea solution should give a urea nitrogen of 47 mgm. if the urease is potent.

Preservation of Blood for Urea Estimation.—The urea of the blood does not decrease at room temperature until after eight hours. It may decrease 20 per cent in twenty-four hours. Ice-chest temperature almost wholly prevents any decrease for twenty-four hours. Sodium fluoride interferes with the reaction of the urease ferment and will give erroneous results. This interference may be overcome in bloods containing not over 30 mgm. of sodium fluoride per cubic centimeters by diluting with 10 volumes of water previous to the mixture with urease.[1] If sodium fluoride has been used as a preservative of blood for urea estimation its presence should be stated on the label.

BLOOD UREA RETENTION TEST.[2]

A Simple Method.—Experience with this method at the Brady Urological Institute for one and a half years shows that it is rapid, simple and reliable. For readings below 35 mgm. of urea nitrogen per 100 cc. this mercury combining test is as close as one could obtain using duplicate estimations by the urease method. In the higher values the difference between the mercury combining power and the ureas method is not sufficiently large to be of clinical significance. Less than fifteen minutes are required to do a single test.[3]

Reagents and Apparatus.

Potassium oxalate, 20 per cent solution.
4 ounces 10 per cent trichloracetic acid, 200 cc.
Mercuric chloride, chemically pure, accurate 5 per cent solution.

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[1] Roe *et al.*, Jour. Biol. Chem., 1927, **75**, 685.
[2] Hench and Aldrich: Arch. Int. Med., 1926, **38**, 47.
[3] White and Ricker: Jour. Amer. Med. Assn., 1929, **92**, 1324.

Sodium carbonate, 22 per cent solution.
Hypodermic needle, 22- or 23-gauge.
5- or 10-cc. syringe.
Several test-tubes.
1-cc. and 5-cc. pipets (2 of each), 2 glass slides.
Funnels and filter paper or centrifuge.

The LaMotte Chemical Products Company of Baltimore Md., supply a convenient outfit for this estimation in a box with directions. Their glassware is graduated so that no estimations are necessary. The cost is $15.00.

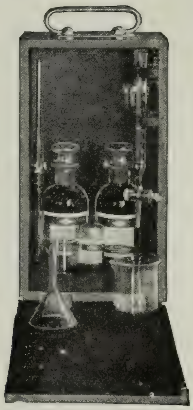

Fig. 34.—LaMotte blood urea outfit.

Principle.—The test depends on the power of mercuric chloride to combine with urea, creatinine and uric acid in a protein-free filtrate, obtained by mixing the blood with an equal part of 10 per cent trichloracetic acid solution and filtering. As mercury is added the union continues until all the urea products have been used up by the combination. After this the excess mercuric chloride remains free in the solution and may be detected by adding a drop of this solution to a drop of saturated sodium carbonate. A dark reddish-brown indicates the presence of free mercuric chloride. The mercury combining power of a protein-free blood filtrate is increased with an increase of the blood urea. Only fifteen minutes are required to do the test and satisfactory clinical results are obtained.

The Test.—Obtain the blood from a prominent vein in the arm as outlined (page 197). Seven cubic centimeters of oxalated blood is necessary, *sodium fluoride cannot be used for this test.* Add this drop by drop to 7-cc. of a 10 per cent trichloracetic acid in a centrifuge tube. The contents of the tube are thoroughly mixed and centrifuged at high for five minutes. Attach a rubber tube to a 5-cc. pipet. This will enable one to watch the tip of the pipet. By suction draw up exactly 5 cc. of the supernatant clear fluid, taking care that the pipet disturbs none of the sediment. Place the 5 cc. of filtrate in a test tube and titrate with 5 per cent mercuric chloride as follows, until the end point is reached; 1.5 cc. of mercury solution can be added at once to represent minimum normal blood urea. After this add from a 1 cc. pipet, 0.1 cc. amounts of mercuric chloride and after shaking the mixture well, remove a small drop of the mixture and add it to a drop of the saturated sodium carbonate on the glass slide. A yellowish precipitate may occur before the reddish-brown color appears. More mercuric chloride must be added to the blood filtrate until a drop of the mixture will produce *a reddish-brown precipitate* within three seconds. Multiply the number of cubic centimeters of 5 per cent mercuric chloride solution used, by 20 to obtain the mercury combining power of 100 cc. of filtrate. Multiply the results by 2 for the dilution of the trichloracetic acid. Normal mercury combining power for each 100 cc. of blood is 70 to 100. Equivalent blood urea equals mercury combining power minus 60 and the blood urea nitrogen is about one-half the blood urea. This method is accurate to within 4 mgm. per 100 cc. of blood. It offers less chance to gross error than other methods and it requires only fifteen to twenty minutes.

Example.—If 5 cc. of the blood filtrate requires 4 cc. of the 5 per cent mercuric solution to titrate to the end-point the mercury combining power of 100 cc. of the filtrate would be 20 multiplied by 4 or 80. Of 100 cc. of blood it would be 80 multiplied by 2 because of the dilution of trichloracetic acid. The probable blood urea would be 160 minus 60 or 100 mgm. for each 100 cc. The blood urea nitrogen would be about 50 mgm. per 100 cc. of blood.

Interpretation.—The urea content of the blood is usually recorded as urea nitrogen, the nitrogen making up approximately one-half the urea. The readings are slightly higher after a meal rich in proteins and the optimum time to do the test is before breakfast.

The normal urea nitrogen is from 10 to 15 mgm. per 100 cc. of blood although up to 20 does not definitely indicate impaired kidney function. It is a test of glomerular disease rather than tubular. It is low in the tubular degeneration known as nephrosis; also in eclampsia although it is elevated in the nephritis of pregnancy. In acute nephritis the urea nitrogen may go up to 100 for a period of a week and two weeks later may come back to normal as recovery takes place.

In Chronic Renal Disease.—All renal function tests are of most value in chronic disease and blood urea shows an increase only when the chronic disease is of the advanced type. The earliest impairment is shown by the water concentration test. While the water test may show a lack of concentrating power the kidney compensates for this by a relative polyuria and so excretes the necessary urea and other waste products. This state may continue for a long time. Only when the compensating polyuria fails does the blood urea increase.

Experimentally two-thirds of the working kidney substance which is the normal reserve may be removed without the blood urea increasing. When the last third becomes impaired, the blood urea nitrogen shows increased readings and the blood-pressure is usually up. Cardiac failure produces a very abnormal result in the dye test or the water-dilution test. It has relatively little influence on the blood urea and water-concentration tests.

In chronic nephritis, according to Widal, a blood urea nitrogen of 25 to 50 indicates a serious prognosis but prolonged survival is possible. With 50 to 100 mgm. life rarely extends more than a year. With 100 to 150 mgm. life extends from a few weeks to a few months; with over 150 mgm. patients live not longer than a few days.

A reduction of the protein intake definitely aids in reducing the blood urea nitrogen retention.

In Genito-urinary Surgery.—A high blood urea nitrogen in prostatic or other urologic obstruction has not such a serious prognosis as in chronic nephritis. A patient with obstruction to the urinary outflow may have a blood urea nitrogen over 100 and not appear ill. When the obstruction is relieved the blood urea nitrogen rapidly falls to normal if there is no chronic nephritis. The presence of urinary casts suggests nephritis. Patients having a blood urea nitrogen of over 30 mgm. should be regarded as unfavorable risks for an extensive surgical operation.

In Intestinal Obstruction and Dehydration.—In intestinal obstruction, whether of the mechanical or of the adynamic type that occurs in acute general peritonitis, the blood urea nitrogen is increased to four to five times its normal although the kidney function is good. This is due to increased tissue destruction and the depleted state of the body fluids do not allow active renal excretion. Where intestinal obstruction, general peritonitis, severe vomiting or diarrhea is the cause of a high blood urea, the urine is diminished and has a specific gravity over 1.030. Where renal disease is the cause of urea retention the urine specific gravity is below 1.025, usually

1.020 or less, and even the diuresis cannot compensate for the lack of concentrating power in the kidney. The specific gravity of the urine is an important means of distinguishing as to whether a high blood urea is due to intestinal upset or renal failure.

As the classical symptoms and signs frequently do not appear until late in the course of the disease a blood urea estimation is indicated where there is any suspicion of intestinal obstruction.

Recently a protein tolerance test has been used to estimate liver function. The patient is given a gram of protein for each kilo of body weight and the blood urea estimated, at the beginning of the meal, after one and two hours. When the liver function is impaired there is no rise in the blood urea nitrogen following the meal.

Other Non-protein Substances.—The total non-protein nitrogen content of the blood is about 20 to 40 mgm. per 100 cc. About one-half of this is made up of urea nitrogen. Occasionally the total urea content of the blood is reported instead of only its nitrogenous portion. It is important to distinguish these because the urea nitrogen is about one-half (exactly twenty-eight-sixtieths) of the urea. So that a patient having a blood urea nitrogen of 14 would have a *blood urea* of 30. As well as the urea nitrogen the uric acid, ammonia, amino-acids, and creatinine are included in the total non-protein nitrogen. The remaining two-fifths are the other non-protein substances, the chemical nature of which has not been as yet determined. The urea nitrogen is decreased and the undetermined nitrogen increased in eclampsia. Otherwise the estimation of the blood urea nitrogen has the same clinical significance as the estimation of the non-protein nitrogen.

ESTIMATION OF CREATININE.

Reagents.

(*a*) Standard creatinine solutions.

Stock solution—to a 100-cc. volumetric flask add 0.1 gm. pure creatinine and 80 cc. of 0.1 normal hydrochloric acid, then fill with distilled water to the 100-cc. mark.

(*b*) Standard solution.

Six cubic centimeters of the above creatinine stock solution made up to a liter with tenth-normal hydrochloric acid. Add 3 drops of toluene or xylol as a preservative. Five cubic centimeters of this solution contains 0.03 mgm. of creatinine.

Saturated aqueous solution of purified picric acid. Two hundred cubic centimeters (2 gm. per liter).

Ten per cent sodium hydroxide solution 100 cc.

The Test.—Mix 25 cc. of the picric acid solution with 6 cc. of the 10 per cent sodium hydroxide solution immediately before each test. Put the protein-free filtrate (see page 137) into one tube and the standard into another tube as follows:

Blood filtrate.	Standard.
10 cc. filtrate	5 cc. standard creatinine solution (*b*)
5 cc. alkaline picrate	15 cc. distilled water
	10 cc. alkaline picrate

Add the alkaline picrate to each about the same time and after ten minutes compare in a colorimeter. Do not take more than five minutes for this estimation.

Calculation.—Using a cup and plunger colorimeter set the unknown at 30. Then the reading of the standard divided by 20 gives the milligram of creatinine per 100 cc. of blood.

Interpretation.—Normal 1 to 2 mgm. per 100 cc. Reading of 4 or over in chronic disease indicates such a severe impairment that the patient will not live a year. Creatinine is easily excreted and only when the kidney is badly damaged is it held back. This point makes its estimation valuable in late prognosis. The test need only be done when the blood urea nitrogen is elevated. In nephritis with casts the blood creatinine will be about 4 mgm. when the urea nitrogen will be 100 mgm., whereas in a urological patient the blood urea nitrogen will reach 150 mgm. before the creatinine reaches 4 mgm.

In nephritis the degree of anemia is remarkably parallel to creatinine accumulation. A hemoglobin below 50 per cent and a red cell count below 3 million having much the same significance as a creatinine over 5 mgm. The exceptions to this parallelism occur when a patient with nephritis has had a hemorrhage when the anemia will be unduly marked. If the patient has had heart failure early in the course of the disease the anemia is late in appearing.

BLOOD URIC ACID.

This estimation requires much time and apparatus and as there is little information to be gained by doing it, the technique is not outlined here. It is very clearly described in any of the text-books on practical physiological chemistry.

Interpretation.—According to the new methods of Folin and Benedict, 1923–1925, 3 to 4 mgm. is the normal amount of uric acid in 100 cc. of blood. Over 5 mgm. in a patient who is free from nephritis strongly suggests gout. Uric acid retention is very early in nephritis because this substance is much more difficult to excrete than urea and so is retained with a much slighter renal impairment than is urea. A purin-free diet (milk), the administration of cinchophen or salicylates lower the blood uric acid in gout. A careful search at the outer margin of the helix of the ear for tophi containing the needle-shaped crystals of sodium monourate is a much sounder diagnostic method for gout than the uric acid content of the blood. This is especially true of patients past middle age who have hypertension and probably enough renal impairment to raise the blood uric acid content without showing any other disturbances.

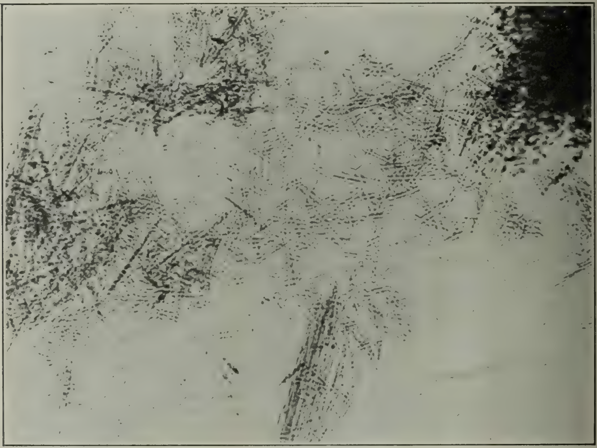

Fig. 35.—The linear crystals of sodium mono-urate × 500 removed from a tophus at the outer margin of the helix of the ear.

BLOOD CHOLESTEROL.

Many interesting studies concerning the rôle played by cholesterol in the animal body have been made and may some day prove valuable. At present they cannot be utilized to advantage in diagnosis and the technique of the test, which is time-consuming, is therefore not described here. It may be found in any text on practical physiological chemistry.

Interpretation.—Normal 150 to 190 mgm. per 100 cc. of blood. The reticulo-endothelial system in the bone-marrow, spleen and liver plays a large part in cholesterol metabolism. Disease affecting this system such as anemias and leukemias also disturb cholesterol metabolism. Actively growing cells will use up cholesterol. Low blood cholesterol may be obtained by increasing the activity of the reticulo-endothelial system of cells.

The cholesterol content of the blood is temporarily lowered by diets that are poor in lipoids, such as the high starch diet. Values lower than normal occur in high fevers and frequently but not always in pernicious anemia. The cholesterol content is increased temporarily by a diet excessively rich in lipoids such as butter, eggs and meat. It can likely be synthetized in the body from fats but its combustion in the body is improbable. Normal persons have a higher reading in summer than in winter. A physiological increase occurs in pregnancy. Values up to twice the normal occur in chronic tubular and acute nephritis, the higher values being present in cases of pure nephrosis, probably to maintain osmotic pressure since there has been much loss of blood serum albumin through the kidneys. Myxedema and the early stages of malignant disease show an increase up to one and a half times the normal values. Uncomplicated cholelithiasis does not show any constant increase of blood cholesterol but biliary obstruction does.

There is an increase of blood cholesterol in diabetes if the fat metabolism is inadequate and this is a valuable index in the prognosis. If increased readings persist despite diet regulation and insulin, the prognosis is serious and the patient's management requires close watching. Readings up to twice normal may occur and in the lipemia of diabetes the readings may be even ten times the normal. Insulin administration rapidly reduces this accumulation.

Cholesterol which is insoluble in water exists in the bile where it is mostly free and in the red corpuscles where it is free and its content relatively constant in health and disease. In the blood plasma much of the cholesterol is in close association with the proteins and a plasma containing a high percentage may be quite clear. Estimations of the plasma or serum cholesterol are of more clinical value than those of total blood.

INORGANIC BLOOD PHOSPHATES.

Blood Phosphorus.—**Technique.**—Dry clean tubes and syringe, 15 mgm. of potassium oxalate per 100 cc. of blood and immediate chemical analysis are essential.

Interpretation.—The blood phosphorus usually referred to is inorganic phosphate which varies in health between 2.5 and 3.5

mgm. per 100 cc. of blood. Where ossification is active in children readings up to 5 mgm. are found. Values are frequently higher in summer than in winter and in active rickets readings as low as 1.5 mgm. occur. Low readings are sometimes encountered where the bones become decalcified as in osteitis fibrosa, a condition which may be associated with hyperparathyroid activity and hypercalcemia.

De Wesselow advises the estimation of inorganic phosphorus content of blood plasma as an index of renal function. In severe nephritis the increase is somewhat later than that of the blood urea nitrogen but earlier than the creatinine. In nephritis with no nitrogen retention the phosphate is normal. Readings of over 8 mgm. indicate a fatal termination within a month. In uremia or very severe nephritis readings may reach 20 mgm. per 100 cc.

The organic phosphorus is about 24 mgm. per 100 cc. of blood and is largely in the corpuscles. An enzyme phosphatase controls its mobilization. The lipin phosphorus is about 12 mgm. per 100 cc. of blood and is contained in lecithin and cephalin. The lipin in the plasma varies greatly in disease much in the same direction as cholesterol. Phosphorus absorption depends on the diet, the reaction of the intestinal contents (acid reaction favors phosphorus absorption) and antirachitic vitamin; its excretion depends on the activity of the kidneys.

For further study of phosphorus metabolism see Hunter; Goulstonian Lectures.[1]

BLOOD CHLORIDES.

The technique employed[2] for this test requires much practice and time. The blood must be collected under liquid petrolatum to avoid the loss of carbon dioxide from the plasma to the air which in turn will cause a dialysis of chlorides from the cells to the plasma.

Interpretation.—Normal whole blood contains 450 to 500 mgm. of sodium chloride per 100 cc. Normally the plasma contains 570 to 620 mgm. and the cells 260 to 320 mgm. There is a decrease in both plasma and cell chlorides in acute intestinal obstruction,[3] and intravenous sodium chloride is beneficial.

In fevers such as pneumonia there is a decrease due to temporary chloride retention in the tissues. The values are low in diabetes. The figures above normal are found in nephritis involving the tubules, in cardiac failure, eclampsia and prostatic obstruction. This is due to increased amounts in the plasma.

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[1] Lancet, April 26, May 3 and 10, 1930, vol. i.

[2] Van Slyke: Jour. Biol. Chem., 1925, **58**, 1523.

[3] Haden and Ora: Surg., Gynec. and Obst., 1923, **37**, 465.

Blood Estimations Indicated in Various Conditions, Normal and Some Pathological Figures.

Condition.	Sugar.	Cholesterol, per cent.	Chlorides, per cent.	Creatinin, mgm. per 100 cc.	Urea nitrogen, mgm. per 100 cc.	Uric acid, mgm. per 100 cc.
Normal	0.08–0.12	0.150–0.165	0.437–0.475	1–2.5	10–20.	1–3.
Renal diabetes . .	0.11	0.15–0.20				
Mild diabetes . . .	0.16–0.20	0.15–0.20				
Severe diabetes . .	0.20–0.8	0.20–0.82	.....	...	18	
Gout	...	.....	.....	...	12	4–10
Early interstitial nephritis	...	.....	.....	2–4	20–30	3–10
Late interstitial nephritis	...	0.16–0.28	.....	4–35	30–300	10–25
Chronic parenchymatous nephritis . .	...	.....	.....	...	30–60	
Acute nephritis . .	...	.....	0.585	3–6.1	30–100	3–11.4
Arteriosclerosis . .	...	0.20–0.23	.....	...	10–40	3–10
Prostatic obstruction .	...	.....	.....	...	20–46	3–9
Pneumonia . . .	...	.....	.....	...	10–25	
Eclampsia	...	0.23				
Intestinal obstruction, peritonitis . . .	...	.....	.....	...	20–150	

TOTAL BLOOD SERUM CALCIUM.

Principle.—When ammonium oxalate is added to blood serum the oxalate unites with the calcium in the serum to form calcium oxalate. It is dissolved in sulphuric acid and by titrating with hundredth-normal potassium permanganate the amount of calcium present is determined.

Collection of Specimen.—All glassware should be chemically clean. Tap-water sometimes contains traces of calcium which if it is used on glassware will vitiate the results. Test-tubes and syringes should be cleaned with acid, well rinsed several times with distilled water and dried. As the test is performed on serum *no anticoagulant* should be added. Withdraw 10 cc. of blood from a vein. Place in a tube and after the clot contracts, pipet off 2 cc. of serum for the test. The Clark-Collip[1] modification of the Kramer-Tisdall method is most generally used.

Interpretation.—The normal blood serum calcium is 9 to 12 mgm. per 100 cc. of blood. Toward the end of pregnancy there is a slightly lower reading normally. Although the normal reading is at the upper limits of normal in children it is frequently decreased in March and April. Rickets is more prevalent during these months also. Abundant calcium diet and cod-liver oil or sunlight will increase the blood calcium when it is low in rickets. Tetany convulsions do not occur until the blood calcium has dropped to 5 or 6 mgm. After parathyroidectomy the blood calcium will drop to very low figures and tetany develop. In severe chronic nephritis the blood calcium is lowered to nearly one-half that of normal but no tetany occurs. Several 5-gm. doses of calcium chloride taken by mouth at hourly intervals will increase the blood calcium by 1 or 2 mgm. for a few hours. Parathyroid extract (Collip) will produce a more marked increase lasting twelve to twenty-four hours.

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[1] Clark and Collip: Jour. Biol. Chem., 1925, **63**, 461.

Hypercalcemia may occur rarely in hyperplasia or tumors of the parathyroid gland. The total serum calcium is over 13 mgm. per 100 cc. of blood. This calcium is drained from the bones and may give rise to a generalized osteitis fibrosa. Removal of the diseased parathyroid allows the blood calcium to return to normal and the state of the bones immediately improves.

Considering the importance of calcium in the body economy one would expect much valuable information from its estimation. The practical information derived from this test is very scanty when checked against such a well-known clinical sign of calcium deficiency as Chvostek's. This sign is always positive in tetany and will be found positive before the blood calcium is diminished.[1] It is elicited by tapping just in front of the ear cavity and a positive reaction consists of a sudden contraction of the forehead, eyelid, cheek, nose and lips. The other physical signs of tetany are also present before the blood calcium is lowered. With these signs positive a quantitative estimation will show the degree of deficiency. No blood calcium deficiency is present in tuberculosis, asthma, skin eruptions, purpura, hemophilia, epilepsy or chronic arthritis. Some 200 estimations of blood calcium at the Mayo Clinic by Boothby, on patients suffering from ununited fracture showed no calcium deficiency. There is therefore no indication for calcium therapy in these diseases. An estimation of the diffusible blood calcium as opposed to the total calcium will probably prove valuable, but as yet, there is no test suitable for routine estimation. Sixty per cent of calcium is in diffusible form which supplies the free ionized calcium. The 40 per cent of nondiffusible calcium is in combination with protein.

Symptoms of calcium deficiency appear when the amount of diffusible calcium is low or the ionization of it interfered with. Acidosis aids ionization; alkalosis impedes it. So tetany may be produced at times independently of the total calcium level. For further study of calcium metabolism see Hunter. Goulstonian Lectures.[1]

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[1] Sexton: The Diagnostic Value of Blood Calcium Estimation, Med. Clin. North America, September, 1927, p. 603.

BLOOD PROTEINS.

Blood proteins and their fractions are determined by estimating their nitrogen content by means of a micro Kjeldahl method. This requires a fume chamber, considerable time and a ready acquaintance with the procedure. Estimations of the serum protein may be most readily made by the immersion type of Pulfrich's refractometer. *The total plasma protein* is determined by centrifuging the cells of an oxalated blood which does not clot and determining the nitrogen content of the plasma. This figure multiplied by 6.15 gives the protein value. Normal 6.8 to 8.8 per cent. Increased in dehydration and decreased in anemia and in nephritis with edema.

The Total Serum Protein.—After the blood has clotted the serum is used to estimate its nitrogen content. The normal is 6.5 to 8.2 per cent. The values vary as does plasma protein. Fibrinogen may be estimated as the difference between the plasma and serum protein, *i. e.*, the protein content of the clot network or if the fibrinogen be precipitated by calcium chloride then an estimation of the filtrate protein content subtracted from the protein content of the original plasma will give the *fibrinogen* value. Normal 0.3 to 0.6 per cent. It is increased in pneumonia or septicemias but decreased in liver disease and severe anemias.

Serum albumin may be determined on the filtrate after the globulin is precipitated by 22.2 per cent sodium sulphate at 37° C. from the serum. Normal 4.6 to 6.7 per cent. Increased in muscular activity, low in nephrosis.

Serum globulin in serum is obtained by subtracting the albumin figure from the total protein in plasma, the fibrinogen and albumin must be subtracted from the total protein. Normal 1.2 to 2.3 per cent. Increased in uremia, nephrosis, pneumonia and syphilis.

DETERMINATION OF CARBON MONOXIDE HEMOGLOBIN.

The Tannin Test.—Add 1 cc. of water to each of 2 small test-tubes and in each place 4 drops of the blood to be tested. Change the solutions to large test-tubes and add to each 1 cc. of 10 per cent solution of potassium ferricyanide. Shake one of the test-tubes vigorously striking it against the palm of the hand for five minutes. Add 0.5 cc. of yellow ammonium sulphate and 10 cc. of a 10 per cent solution of tannin to each test-tube. A turbid olive-green precipitate will appear in the tube which was shaken and a bright red precipitate in the one which was not shaken and still contains carbon monoxide hemoglobin.

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[1] Lancet, April 26, May 3 and 10, 1930, vol. i.

Interpretation.—The diagnosis of carbon monoxide poisoning is more important now than it was formerly on account of the large numbers of motors used. It is one of the hazards of modern city life. While the chief source is the exhaust gas from gasolene motors in enclosed garages, other occasional sources are from defective furnaces, stoves and ordinary illuminating gas. Acute poisoning is readily recognized but the symptoms of chronic poisoning by small amounts such as a policeman might acquire on a narrow street with a busy traffic, should not be overlooked. Frequently headaches and being easily fatigued are the only complaints. After a patient has breathed pure air for several hours most of the carbon monoxide in the blood disappears although the symptoms may remain for an indefinite time. The chemical tests are more satisfactory and sensitive than the spectroscopic tests which show characteristic color bands only when 25 per cent of saturation is present.

METHEMOGLOBIN ESTIMATION.

When a large amount of methemoglobin is present the blood has a definite chocolate-brown color. For preliminary diagnosis spread the blood blotting paper and compare with normal blood similarly spread.

A spectroscope is necessary for the detection of small amounts. Dilute some blood, one in ten with water, and have it examined spectroscopically for the characteristic bands.

Interpretation.—Methemoglobin poisoning is due to taking large doses of acetalinid or phenacetin over a long period of time. Nitrites will occasionally produce this state and it may occur in shoe-cleaners using a shoe dye, containing nitrobenzol. In fact a few have developed the methemoglobin poisoning by wearing freshly dyed shoes. It is characterized clinically by a marked cyanosis although the blood count is normal.

CHOICE OF TESTS FOR KETOSIS, ACIDOSIS OR ALKALOSIS.

INDICATIONS.

Acidosis.	Alkalosis.
Dehydration.	Vomiting.
Acetone odor on breath.	Excessive alkali ingestion.
Glycosuria.	Tetanic spasms.
Diarrhea.	
Severe nephritis.	
Methyl alcohol poisoning.	
Diabetes.	

CLINICAL TESTS.

Ketone acids.	Alkaline urine.
Tolerance to alkalies.	Tolerance to acids.
CO_2 of alveolar air.	

LABORATORY TESTS.

Blood plasma CO_2 combining power under 50.	Blood plasma CO_2 combining power over 70.
pH under 7.3 in uncompensated acidosis.	pH over 7.45.

TREATMENT.

Treat primary disease.	Treat primary disease.
Water.	Water.
Sugars.	Acids.
Alkalies.	
Insulin in diabetes.	

ALKALI RESERVE AS DETERMINED BY THE CARBON DIOXIDE CAPACITY OF BLOOD PLASMA.

Principle.—The carbon dioxide content of blood plasma is determined by adding to the plasma a strong acid and creating a partial vacuum to liberate carbon dioxide gas, which is then measured in cubic centimeters at normal air pressure. This is recorded in cubic centimeters per 100 cc. of blood plasma (volume per cent).

Apparatus.—Although the Van Slyke apparatus gives the most accurate results, its cost and size limits its use to the laboratories of the large hospitals. The simplified apparatus devised by Fradkin[1] is portable, not very expensive and easily manipulated. It will give results accurate within two points of the Van Slyke apparatus.

The simplified apparatus is manufactured by the Emil Greiner Co., 55 Vandam St., New York City and costs about $33. It consists of a 25-cc. chamber with one stopcock at the top and another at the side. The top 0.5 cc. of the upper stem of the chamber is divided into 0.01-cc. divisions and there is a side arm attached to the lower stopcock for the reception of plasma and reagents. The bottom of the apparatus is connected by a thick rubber tube to a levelling cup filled with mercury. The stopcock at the top opening is used for the removal of fluids from the apparatus. The entire apparatus is well mounted in a wooden case and can be elevated to any desired level by sliding the metal tube, which is held by a thumbscrew, up and down on the metal rod.

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[1] Jour. Lab. and Clin. Med., 1929, **14**, 973.

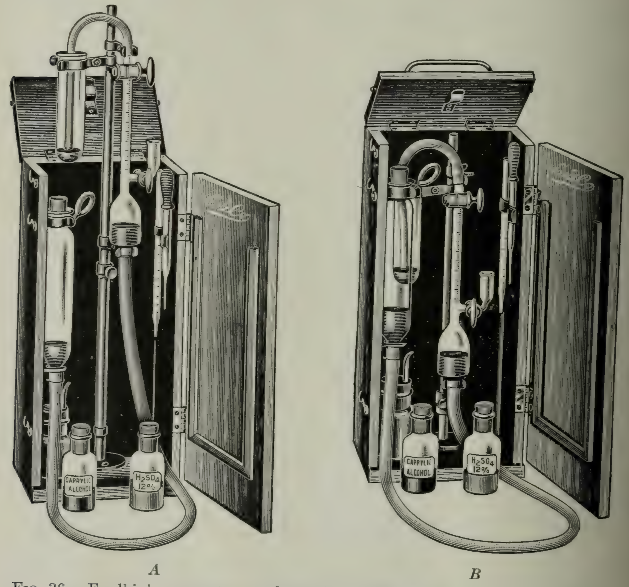

FIG. 36.—Fradkin's apparatus to determine alkali reserve by carbon dioxide capacity of blood plasma.

Reagents.—Twenty per cent potassium oxalate 1 ounce.
12 per cent sulphuric acid 1 ounce.
Caprylic ethyl alcohol 1 ounce.
Distilled water 16 ounces.
Redistilled mercury 50 cc. ($1\frac{1}{2}$ pounds).
Stopcock grease.

Obtaining the Blood for Examination.—Place 4 drops of 20 per cent potassium oxalate in a small test-tube fitted with a cork. Withdraw about 5 cc. of blood from the vein at the elbow. Pour it into the tube containing the oxalate. *Cork immediately* and invert the tube several times. Allow the plasma to separate by gravity while testing the apparatus.

Testing the Apparatus.—It is essential to have both stopcocks absolutely air-tight. If they do not fit well they should be reground by using a small amount of jeweller's rouge and turning them for ten minutes. After this they should be lubricated with a mixture made by dissolving, by the aid of heat, 1 part of rubber in 3 parts of lanolin. Elevate the apparatus to position *A*. by means of the thumb-screw on the still rod and tube. Raise the levelling cup slowly so that a little mercury flows into the side arm and close the lower stopcock. Still elevate the levelling cup so that the mercury flows through the upper stopcock. Then close it. Produce a vacuum by lowering the mercury levelling cup. Shake the levelling cup slowly up and down to determine if the apparatus is air-tight. A gas-free air-tight apparatus will cause the mercury to strike the upper cock with a sharp click. If this click is not heard it will be necessary to determine the source of the air leak and correct it by further greasing of the cocks or grinding, if necessary.

The Test.—Fill the chamber with mercury. Make sure that it is gas-free. Introduce 0.5 cc. distilled water into the side receptacle with a pipet. Transfer 0.5 cc. of blood plasma with a pipet into the side receptacle and place it *under* the surface of the water. With the mercury levelling-cup in position *A* rotate the stopcock cautiously so as to allow all the fluid but no air to enter the chamber. Run a small drop of caprylic alcohol into the side-arm to prevent foaming and finally, carefully run in 0.2 cc. of 12 per cent sulphuric acid to displace the CO_2, taking care that no air gets in also. Seal the stopcock by running a drop of mercury into the side receptacle. Lower the mercury levelling-cup until the mercury in the chamber reaches mark 25. Slowly shake the box back and forth for two minutes to agitate the solution in the chamber and thus insure complete extraction of the carbon dioxide. The levelling-cup is then raised rapidly with the left hand so that it reaches the level of the mercury meniscus in the stem. The volume of gas is read off at once in order to avoid the absorption of carbon dioxide. The results are calculated for 100 cc. of plasma by use of the following table on page 162.

TABLE FOR CALCULATION OF CARBON DIOXIDE CAPACITY OF PLASMA.[1]

(In cubic centimeters of CO_2 chemically bound by 100 cc. of plasma.)

Stem Reading.	Vol. per cent of CO_2.	Stem Reading.	Vol. per cent of CO_2.	Stem Reading.	Vol. per cent of CO_2.
0.100	9.1	0.235	35.2	0.370	61.3
0.105	10.1	0.240	36.1	0.375	62.3
0.110	11.0	0.245	37.1	0.380	63.2
0.115	12.0	0.250	38.1	0.385	64.2
0.120	13.0	0.255	39.1	0.390	65.2
0.125	13.0	0.260	40.0	0.395	66.1
0.130	14.9	0.265	41.0	0.400	67.1
0.135	15.9	0.270	42.0	0.405	68.1
0.140	16.8	0.275	42.9	0.410	69.0
0.145	17.8	0.280	43.9	0.415	70.0
0.150	18.8	0.285	44.9	0.420	71.0
0.155	19.7	0.290	45.8	0.425	71.9
0.160	20.7	0.295	46.8	0.430	72.9
0.165	21.7	0.300	47.5	0.435	73.9
0.170	22.6	0.305	48.7	0.440	74.8
0.175	23.6	0.310	49.7	0.445	75.8
0.180	24.6	0.315	50.7	0.450	76.8
0.185	25.5	0.320	51.6	0.455	77.8
0.190	26.5	0.325	52.6	0.460	78.7
0.195	27.5	0.330	53.6	0.465	79.1
0.200	28.4	0.335	54.5	0.470	80.5
0.205	29.4	0.340	55.6	0.475	81.6
0.210	30.3	0.345	56.7	0.480	82.6
0.215	31.3	0.350	57.4	0.485	83.6
0.220	32.3	0.355	58.4	0.490	84.5
0.225	33.2	0.360	59.4	0.495	85.5
0.230	34.2	0.365	60.3	0.500	86.5

[1] Fradkin: Jour. Lab. and Clin. Med., July, 1929, p. 977.

Thus if the reading of the gas volume on the graduated stem be 0.350 there will be 57.4 cc. of carbon dioxide chemically bound by 100 cc. of plasma.

Normal—50 to 65 cc. of carbon dioxide chemically bound by 100 cc. of plasma.

Cleaning the Apparatus.—The chamber is cleaned with two washings of distilled water, about 5 cc. each, by admitting through the side arm, raising the levelling-cup and expelling through the upper stopcock. After considerable mercury has run through into the test-tube at the top it may be washed and replaced in the levelling-cup.

Repeat the test several times using normal blood to become acquainted with the technique and to obtain satisfactory results when an estimation is urgently needed.

Interpretation.—The carbon dioxide combining-power of plasma indicates the alkaline reserve of bicarbonates in the body fluids. Readings of 50 to 30 volumes per cent indicate a lowered alkali reserve but, because of the buffer substances in the blood, there will not likely be any change in the hydrogen-ion concentration. This is termed a compensated acidosis.

Readings below 30 volumes per cent indicate very little alkaline reserve of bicarbonate, As there is relatively more acid substance the hydrogen-ion concentration is decreased, producing an uncompensated acidosis.

In diabetes the blood sugar is usually 0.3 per cent or more and diacetic is always present in the urine before the carbon dioxide combining-power goes below 50 volume per cent. Drowsiness only appears when the readings reach 30 or lower.

When equal changes in the readings are due to drugs producing acidosis the symptoms are very slight.

Readings over 70 volumes per cent are found in alkalosis. For further considerations see pages 168 and 171.

HYDROGEN-ION CONCENTRATION OF THE BLOOD PLASMA. (CULLEN'S METHOD.)

Principle.—Blood collected with CO_2 loss and diluted with saline containing phenol red indicator develops a color shade depending on its hydrogen-ion concentration. Matching this color with standards will determine the pH of the blood.

The Test.—This test is not in common use because it detects only very advanced uncompensated changes in the acid-base equilibrium. The La Motte Chemical Company market an outfit with standards and directions for estimating the pH of blood.

Interpretation.

Normal 7.3 to 7.5.

A pH below 7.3 indicates uncompensated acidosis and above 7.5 indicates uncompensated alkalosis. Because of the buffer action of the salts and protein in the blood its pH is remarkably constant even when there is a change in the alkali reserve as indicated by the CO_2 combining power. Drugs referred to under acidosis and alkalosis (page 171) can produce changes in pH with fewer symptoms than occur with similar pH changes in the diseased states.

THE DETERMINATION OF ALKALI RESERVE BY ESTIMATING THE ALVEOLAR AIR CARBON DIOXIDE TENSION. (MARRIOTT'S METHOD.)

Note.—This is a test done on expired air, but because this air is in equilibrium with venous blood in the pulmonary capillaries the CO_2 percentage or partial pressure is approximately that of venous blood.

Apparatus.—The Marriott apparatus which consists of a rubber football bag, a bottle of standard bicarbonate solution with phenolphthalein added, a set of eight standard colors which represent the result of CO_2 tensions varying from 10 to 45 mm. of mercury on the standard solution. It can be obtained from Hynson, Westcott & Dunning, Baltimore, Md.

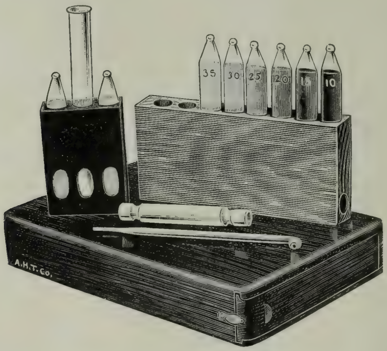

FIG. 37.—Marriott's apparatus for estimating the CO_2 content of alveolar air.

Obtaining the Specimen.—About 600 cc. of air are blown into the rubber bag with an atomizer bulb, and the rubber tube compressed. *The patient should be at rest and breathing naturally.* At the end of a normal inspiration the patient takes the tube in his mouth and breathes four times, emptying the bag at each inspiration, his nostrils being held closed. Then the tube is pinched off and the air analyzed *immediately.* In the case of comatose patients, five breaths are taken instead of four.

The Test.—Two or 3 cc. of the standard bicarbonate solution are poured into a clean test-tube of the same diameter as the tubes containing the colored standard solutions. Air from the bag is then bubbled through the solution by means of a glass tube drawn out to a fine capillary point until the solution is saturated, as shown by the fact that no further color change occurs. The color is immediately compared with that in the standard tubes.

Interpretation.—*Normal* 40 *to* 45 *mm.*—More than one determination should be made in every instance, especially in confirmation of a low tension, since errors in technique of collection of sample lead to too low rather than too high results.

Tensions between 30 and 35 are indicative of a mild acidosis. When the tension is as low as 20 mm. the individual is nearing the comatose state. In coma associated with acidosis the tension may be as low as 8 to 10 mm. In infants the CO_2 tension is normally 5 mm. lower than in adults.

Sources of Error.—Changes in the pulmonary epithelium such as would prevent the air in the lungs from coming in equilibrium with the blood in alveolar air. For this reason one is hardly justified in drawing conclusions regarding acidosis from the composition of the alveolar air in patients with pulmonary affections. In such cases the test for tolerance to alkalies or the CO_2 combining power of blood plasma is indicated.

ALKALI TOLERANCE.

PRINCIPLE.—Small doses of sodium bicarbonate will turn the urine alkaline in normal individuals. In acidosis, large doses are required. If there is marked renal deficiency the urine may not become alkaline. A quick change to alkaline is certain evidence of the absence of acidosis. A persistently acid reaction after continued alkali ingestion does not always indicate the degree of acidosis because kidney excretion may not be satisfactory.

The Test.—Give 5 gm.[1] of sodium bicarbonate in a glass of water for an adult patient. Repeat the dose every half hour until the urine becomes alkaline, making the test for reaction with litmus or phenolphthalein before each dose is given. If the urine reaction is indefinite, boiling will make it more distinct by changing the bicarbonate to carbonate. Note the number of grams necessary to obtain an alkaline reaction.

Interpretation.—In a normal adult 5 to 10 gm. of sodium bicarbonate will render the urine alkaline. If the patient is not of average size 0.5 gm. (8 grains) per kilo ($2\frac{1}{5}$ pounds) of body weight may be taken as a maximum to produce the alkaline reaction when no acidosis is present.

In mild acidosis 25 gm. of bicarbonate may be necessary to alkalinize the urine, the first part being taken up to replace deficient alkali reserve in the body fluids before any alkali is excreted by the kidney. When acidosis is severe it may require 1 gm. or more of sodium bicarbonate per kilo of body weight, or for an average sized adult over 60 gm. to turn the urine alkaline. It is wise not to persist with large and frequent doses of alkali once the diagnosis is established as an alkalosis might result if the kidney excretion is badly deranged.

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[1] Approximately a large teaspoonful, level measure.

DIAGNOSIS OF ALKALOSIS BY THE URINE REACTION.

In alkalosis the urine is persistently alkaline if renal excretion is active and adequate whereas normally it is acid in reaction except for a short time following a heavy meal. In making a diagnosis of alkalosis from the urine reaction, it will be necessary to make sure that the urine is fresh, and that there is no prostatic obstruction or alkaline cystitis.

As therapy for the alkalosis, removing the cause and giving an adequate supply of water are of greatest importance. Maximum doses of the drugs which produce lowering of the hydrogen-ion concentration may assist in correcting alkalosis.

ACIDOSIS.

Diagnosis of Acidosis.—Only when acidosis is advanced can it be recognized clinically. The characteristic acetone odor on the breath is the most trustworthy sign. Rapid breathing may be present and if the patient be conscious he may complain of headache. To detect acidosis early laboratory tests are a necessity. The comparative values of four tests are given in the following table.

Relationship of Tests for Acidosis and Alkalosis.[1]—The CO_2 combining power of blood plasma is the most reliable test. The other indirect tests have an approximate relationship and may occasionally be in error.

		Acidosis.		
Tests.	Normal values.	Mild.	Severe.	Alkalosis.
Blood plasma CO_2 Combining power, vol. per cent	50–80	30–50	Below 30	80 or more
Alveolar air CO_2 tension (Marriott)	35–45	25–35	Below 25	40–45
Acetone bodies in urine, for ketosis only	0	Positive ferric chloride and sodium nitroprusside reactions	Positive ferric chloride and sodium nitroprusside reactions	0
Alkali tolerance; sodium bicarbonate required to turn urine alkaline:	. . .	. . .	. . .	Urine is continually alkaline if excretion is good.
(*a*) Grams per kilo of body weight	0–0.5 gm.	0.5–1 gm.	Over 1 gm.	
(*b*) Approximate grams for adult weighing 60 kilos	0–30 gm.	30–60 gm.	Over 60 gm.	

1 gm. = 15 grains. 1 kilo = $2\frac{1}{5}$ pounds.

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[1] Modified from Van Slyke: Jour. Biol. Chem., 1918, **33**, 271.

DISTURBED EQUILIBRIUM OF BODY FLUIDS.

Acidosis was first recognized in advanced diabetes as the result of ketone acids in the blood and the finding of ketone substances in the urine was then sufficient for a diagnosis of acidosis. We now know that prolonged vomiting results in an alkalosis and that ketone substances may also be present in the urine due to the insufficient carbohydrate nourishment. Van Slyke has shown that acidosis occurs when the absorption or formation of acids in the body exceeds the rate of their elimination. This produces an abnormal state by increasing the hydrogen-ion concentration of the blood and lowers its alkali reserve of bicarbonate below the extreme normal limit.

By the application of physical laws it is possible to determine chemical changes in the tissues by a study of the blood plasma. The fixed base content of the blood is remarkably constant, even more so than the hydrogen-ion concentration and this is important in maintaining osmotic pressure.

The reaction of the normal blood serum in terms of hydrogen-ion concentration is about 7.35. The maximum variations in disease compatible with recovery are pH 7 to pH 8. A pH of 7, which is the absolute neutral point, may be reached in very severe acidosis while the maximum alkaline value may be reached in marked alkalosis. This shows the very small variation of reaction which occurs in human blood. Most tap-waters have a pH of about 7. It is very remarkable that the acid equivalent of several hundred cubic centimeters of concentrated hydrochloric acid is thrown off by the carbon dioxide vapor exhaled from the lungs in twenty-four hours.

The blood is a buffer solution to which one may add moderate amounts of acid or alkali with only a slight change in the resulting hydrogen-ion concentration (acid or alkaline reaction). The most important substances in maintaining hydrogen-ion equilibrium are sodium bicarbonate ($NaHCO_3$) on the alkaline side and on the acid side carbonic acid (H_2CO_3) which results from carbon dioxide (CO_2) in solution. When ample bicarbonate is present the blood is at its best as a buffer solution. Other blood constituents which maintain equilibrium of reaction are phosphates, chlorides, hemoglobin, protein, oxidation of organic compounds, the synthesis of ammonia and the excretion of carbon dioxide from the lungs as well as either acid or alkaline substances by the kidney to maintain equilibrium of the body fluids.

Acidosis is compensated when, with lowered bicarbonate alkali reserve, there is a corresponding decrease in carbonic acid gas leaving the hydrogen-ion concentration unchanged. It is uncompensated when there is more carbonic acid gas than basic bicarbonate to combine with it thus producing an increased hydrogen-ion concentration.

The preservation of a normal osmotic pressure of the blood and tissues, which depends on salts (electrolytes) and non-protein nitrogenous chemicals, is perhaps more important than the hydrogen-ion concentration. The osmotic pressure depends on the chloride and bicarbonate salts of sodium, potassium, calcium and other fixed bases and to a lesser extent on the same basis elements bound to protein, phosphate, lactate and other acids. These salts are electrolytes and contribute 90 per cent of the osmotic pressure of the blood plasma.[1] Non-electrolytes such as non-protein nitrogenous substances (urea) and glucose assist in maintaining the osmotic pressure and their increase in the blood during disease is usually accompanied by a lowering of electrolytes.

Acidosis frequently occurs in:

1. **Severe dehydration.**
2. **Diabetes.**
3. **Nephritis.**

It also occurs in:

1. **Non-diabetic ketosis in childhood.**
2. **Starvation.**
3. **Toxemia of pregnancy.**
4. **Surgical anesthesia.**
5. **Methyl alcohol poisoning.**
6. **Excessive introduction of acids.**

Alkalosis occurs in:

1. **Excessive alkali ingestion.**
2. **Loss of hydrochloric acid by vomiting, gastric lavage or pyloric obstruction.**
3. **Excessive elimination of carbon dioxide by rapid respiration.**
4. **Tetany which is associated with parathyroid deficiency or rickets.**
5. **X-ray therapy.**

Depression of tissue oxidation is a prominent cause of either acidosis or alkalosis.

Dehydration.—Dehydration occurs when there is a rapid loss of water from the body which cannot be adequately compensated by fluid intake. Diarrhea, as it occurs in children is one example. The most marked chemical change is an increase in the blood plasma protein to almost twice its normal amount. Most of the salts and non-protein substances concentrate in a similar manner except the alkaline bicarbonate salts which are lost in the frequent watery stools and replaced by base lactates resulting from decreased circulation and base phosphates which are not adequately excreted by reason of the diminished urinary output. If vomiting occurs there is a loss of hydrochloric acid and a lowering of the chloride salts resulting in an increase in the base bicarbonate giving rise to an alkalosis.

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[1] Marriott and Hartmann: Jour. Am. Med. Assn., 1923, **91**, 1675.

Management.—The first urgent need is water and the next is nutrition which is best supplied by 5 per cent glucose. Where vomiting is present chloride in the form of physiological sodium chloride is indicated. If diarrhea is the leading symptom a 5 per cent sodium bicarbonate solution should be administered rather than the sodium chloride. The necessary amount of these may be determined by the CO_2 combining power of blood plasma or by the reaction of the urine. When possible the oral route should be chosen. In vomiting or diarrhea subcutaneous or intraperitoneal injection must be resorted to. The solution should have the same hydrogen-ion concentration as the blood; namely, 7.4.

If sodium bicarbonate is given it will be necessary to add a drop of phenolphthalein indicator and to bubble carbon dioxide through the solution until the color disappears. Enough of such a fluid to establish a normal blood volume and oxygen inhalation will oxidize and remove any accumulated lactic acid in the tissues.

Diabetes.—That fats burn in the flame of the carbohydrates and if the proportion of carbohydrate combustion be too small the fats smoke to form acetone bodies is an axiom that has stood the test of time. While this may occur in any condition of insufficient carbohydrate nutrition, it is most marked and most constant in diabetes mellitus where the tissues, due to the inability of the pancreas to secrete insulin, are unable to utilize the glucose present in the blood. In diabetes there is a marked diuresis which rids the blood of the accumulated glucose. Such a diuresis also carries out chloride and bicarbonate salts and so lowers the fixed bases; if what remains of the bases is mostly taken up by the ketone acids (diacetic, beta-oxybutric and lactic) a deficient carbon dioxide carrying power and an increase in the pH of the blood will result. Either a hyperglycemia or a glycosuria with ketonic acids is sufficient for a diagnosis. Large doses of sodium bicarbonate by mouth will not turn the urine alkaline.

Treatment.—Insulin, glucose and water are the essentials. If the plasma bicarbonate is low, sodium bicarbonate in addition to the above measures will assist in a rapid return to normal.

Nephritis.—In acute nephritis uremic convulsions may result in an accumulation of lactic acid which will use up the alkaline bicarbonate. Vomiting or diarrhea may produce a condition referred to under dehydration. If edema occurs, there is transudation of the salts and water into the tissues but the osmotic pressure of the blood is maintained at normal by the increase in the urea. In severe chronic nephritis there is a loss of the chloride salts and the fixed bases instead of combining with bicarbonate are neutralized by the acid phosphates and sulphates which are inadequately removed because the diseased kidney is unable to maintain a high urine acidity. The total salt content is low but the total osmotic pressure is normal because of the increase of blood urea. An increase in alkali tolerance is an early sign and in the terminal stages of the disease there will be dyspnea and a lessened carbon dioxide tension of the blood and the alveolar air. The acidosis is approximately equal in degree to the urea retention.

Treatment.—The acidosis does not require active treatment. Adequate fluid intake and a liberal vegetable diet which contains much alkaline ash will suffice. Alkali will temporarily correct the acidosis but has no influence on the course of the nephritis.

Non-diabetic Ketosis in Childhood.—Attacks of drowsiness with vomiting, diarrhea or slight fever and a marked acetone odor of the breath may occur from no apparent cause. There may be more fat in the diet than the child can tolerate. In well-advanced cases there is usually some abdominal distention and the drowsiness may approach coma. The urine contains ketonic acids and it remains acid in reaction even when large doses of sodium bicarbonate are given by mouth. Later the carbon dioxide tension of the blood and alveolar air is lessened.

Management.—A decrease in the fat content of the diet, a generous intake of water and glucose will correct the condition. If diarrhea is a marked feature of the illness sodium bicarbonate may be added to correct a lessened alkali reserve.

Starvation.—A mild acidosis usually starts after a fast of twenty-four hours, followed by the appearance of ketone acids in the urine for three or four days. It is not dangerous to normal persons although it may cause headache and nausea. It disappears when a normal carbonate and water intake is established.

Toxemia of Pregnancy.—There is no definite acidosis in toxemia of pregnancy although due to the lack of nourishment, ketonic acids may be found in the urine. Adequate water and sugar or carbohydrate intake will cause the ketonic acids to disappear from the urine. In eclampsia the carbon dioxide combining power falls to 30 volumes per cent or lower with marked disturbances in the oxidative process which may produce an uncompensated acidosis. This may be the cause or result of the eclamptic outbreak. As treatment 300 cc. of 25 per cent dextrose may be injected intravenously. Some give sodium bicarbonate in addition to the above while others give 1 unit of insulin for each 3 gm. of dextrose. For studies of the intricate problem of acid base equilibrium see Peters, *et al.*[1]

Surgical Anesthesia.—This is accompanied by a slight fall in the blood carbon dioxide carrying power which returns to normal after twenty-four hours and the blood pH which returns to normal in three or four hours. These return more quickly after gas oxygen anesthesia than after ether or chloroform. Ketonuria occurs frequently following operations and occasionally before them due to fasting. Its presence is not proportional to the reduction in alkali reserve. It is only the result of the fasting immediately after the operation and except in diabetes it has no serious clinical significance. It should be treated by appropriate diet and fluid intake. The rapid return to normal of the acid base-balance makes the value of alkalies questionable.

Methyl Alcohol Poisoning.—Methyl alcohol ingestion is attended with the formation of lactic and formic acid in the blood and a fall in the plasma bicarbonates. Alkali therapy will reduce the acidosis.

Carbon monoxide poisoning also causes an acidosis.

Excessive Introduction of Acids.—Hydrochloric acid when given by mouth in large amounts will produce an acidosis. A hydrogen-ion concentration of 7.3 to 7.2 may be induced by giving large doses of orthophosphoric acid, ammonium chloride, calcium chloride or ammonium phosphate.[2] These substances are powerful diuretics.

HYDROGEN-ION CONCENTRATION TOWARD THE ALKALINE SIDE (INCREASED pH).

Alkalosis is due to 1, excessive alkali therapy or ingestion of any salt such as sodium citrate composed of an inorganic base and an organic acid which may be oxidized into carbon dioxide and water. Massive doses of sodium bicarbonate will produce a hydrogen-ion concentration of 7.5. This extreme variation disappears twenty-four hours after the drug is discontinued.

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[1] Jour. Clin. Invest., 1929, **6**, 517.

[2] Koehler, J.: Biol. Chem., 1927, **72**, 99.

2. Loss of hydrochloric acid by vomiting, gastric lavage, or pyloric obstruction.

3. Excessive elimination of carbon dioxide by rapid respiration.

4. Tetany which is associated with parathyroid deficiency, rickets, diminished calcium and increased phosphorus of the blood. There is always an increased alkalinity of the blood.

5. *X*-ray therapy. The alkalosis usually persists for several days.

The symptoms of alkalosis are essentially those of tetany, the earliest being restlessness, tremors and Chvostek's sign. Later typical tetanic spasm occurs. It is severe when the blood reaches a pH of 7.8. The carbon dioxide combining power of the blood is increased to 80 volumes per cent or more. Acetone may be present in the urine which however is *usually alkaline*. As a remedy one of the salts which lessen the hydrogen-ion concentration is of value. Ammonium chloride or dilute hydrochloric acid taken by mouth is best but if this is impossible several hundred cubic centimeters of a 1 per cent ammonium chloride solution in isotonic saline may be *slowly* injected intravenously.

TOTAL BLOOD VOLUME.

Principle.—A colored non-absorbable substance which is non-toxic is injected into a vein and after it has distributed itself evenly throughout the bloodvessels a sample of the blood is withdrawn. By estimating colorimetrically the amount of dye present, the total volume of blood can be determined.

The Test.—Vital red is the dye usually injected. It is not a common test in clinical work but anyone wishing to use it may look up the original article.[1]

Interpretation.—The plasma volume is 5 per cent (range 2.4 to 5.6) and the total blood 8.8 per cent (range 7.8 to 9.7) of the body weight. The method outlined may indicate to a slight extent the lymph volume also. Little or no use is made of this test in clinical diagnosis but it has given us some important information about the body in disease. The usual tests of chemical substances in the blood or cell counts tell only the *concentration* of these substances. They do not give absolute values for these determinations. That is, the ordinary hemoglobin determination does not tell the total oxygen carrying capacity of the blood. Anemias are apparent rather than real when the blood is diluted by an increase in plasma while the cell volume is normal.

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[1] Keith, Rowntree and Geraghty: A Method for the Determination of Blood Volume, Arch. Int. Med., 1915, **16**, 547.

Both volumes are decreased in obesity. In the dehydration fevers, the plasma volume is decreased although the cell volume is increased. A most reliable clinical sign of this condition is a dry tongue which can be seen to moisten with a copious intravenous saline injection. Many anemias have large plasma volumes. In true polycythemia the plasma volume as well as the cell count and cell volume are increased. There is a great increase in the plasma volume in edema. A reduction in blood volume, if present in glomerulonephritis, is usually due to a diminished cell volume indicating actual anemia. In nephrosis without anemia the cell and plasma volumes are normal but where the cells are decreased the plasma volume is definitely increased.

VISCOSITY OF THE BLOOD.

PRINCIPLE.—The viscosity of blood is compared with that of water on the principle that fluids at equal temperature and pressure passing through tubes of equal caliber vary in their rate of flow in direct proportion to their internal friction, that is to their viscosity.

The Test.—The best type of apparatus is that of Hess and it is very simple. Only about three minutes are required to make a test. Complete directions accompany the apparatus. For a good discussion of the subject read Austrian[1] or Bricher.[2]

Normal 4.3 to 5.3 for males.
3.9 to 4.9 for females.
Serum 1.7 to 2 for both sexes.

Interpretation.—The viscosity depends on (1) the cell volume. An increased cell volume increases the viscosity and white cells are more viscid than red cells so that in high leucocytosis or leukemia the viscosity is increased. The cell volume gives a rough idea of the viscosity.

2. The hemoglobin content. An increased hemoglobin content causes an increase in viscosity and a hemoglobin estimation will show in an approximate degree the viscosity present. Cyanosis increases the viscosity and venous blood is more viscid than arterial.

3. The protein content. Dehydration which increases the protein content increases the viscosity. General edema or anemia which lowers the protein content also lowers the viscosity.

In health intense perspiration for a period of an hour without drinking fluids will produce a 20 per cent increase in blood viscosity. In *severe pneumonias* the blood viscosity is greatly increased and so a great load is thrown on the heart muscle. The viscosity is greatly increased in *severe diarrheas* and *prolonged vomiting.* Its estimation is of value to differentiate pelvic inflammations or peritonitis, where it is high, from hemorrhage such as is due to ectopic pregnancy where the viscosity is low. The readings are greatly decreased in anemias.

In an extensive study Hess has shown the relation between the heart work and the red cells. He states that it is not the amount of blood thrown out by the heart but rather the load of cells and hemoglobin it contains that determines the index of effectiveness of the heart's work. A greater number of red cells for each unit of blood volume is more effective than a small number of cells. With the cell increase there is an increase in viscosity which determines the heart's work. The cell concentration and viscosity give optimum efficiency with the minimal heart work when the cell volume lies between 40 and 50 per cent of the blood volume.

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[1] Bull. Johns Hopkins Hosp., 1911, vol. **22**.

[2] Jour. Lab. and Clin. Med., 1921, vol. **7** and 1922, vol. **7**.

RATE OF FLOW OF THE BLOOD.

The rate of bloodflow is estimated by several different methods all of which are highly technical. G. N. Stewart injected hypertonic sodium chloride into one vein and found out when it arrived at another by noting the electric conductivity of the blood at that point. He also used methylene blue injection and noticed when it arrived at the common carotid artery by transillumination. Recently[1] the estimation has been made by injecting a radio-active salt at one point and noting the time of arrival at another point by the use of a complex detecting device. In patients with a normal cardio-respiratory system the blood flowed from one elbow to the other in from fifteen to twenty-one seconds. In cardiac failure the blood took fifty to seventy seconds to flow from elbow to elbow. The vital capacity is first lessened then there is a decrease in the velocity of bloodflow and when cardiac failure becomes advanced there is an increase in pulse-pressure. The rate of flow through the lungs in emphysema is normal.

Interpretation.—Clinically a rapid rate of bloodflow may be inferred from a normal body temperature and a normal skin color. The opposite state, a lessened rate of flow is inferred by the subnormal temperature of an extremity, a cyanosis due to defective CO_2 elimination and edema due to a lack of function of the various tissue cells. While the rate of movement of the blood especially in the extremities is primarily dependent upon the efficiency of the heart and the peripheral vessels it is secondarily dependent upon the blood volume and its viscosity. A lessening of the blood volume with an increase of its viscosity will retard the blood rate with the result that the tissues including the heart muscle itself are undernourished and their waste products only partially eliminated. This causes a general upset of the vital processes which if severe enough may cause death.

Acute Enteritis in an Infant.—The following sample case of acute enteritis in an infant is cited to show the value of a knowledge of the blood volume, viscosity and rate of flow without actually making the determination. In a severe attack the onset of enteritis is sudden with vomiting. There is a high fever and very rapid pulse and often increased respiration. The appearance is drowsy. The eyes are very sunken and have dark circles. The stools are frequent and may contain mucus and blood. Microscopic examination of a stool specimen, obtained by catheter, will show hundreds of polymorphonuclears and lymphocytes in contrast to the fermentative diarrheas where there will be under 10 cells to each high-power field. Each symptom and sign tells something about the disturbance in the metabolic process. The sunken eyes show the water-loss discharged by mouth in vomiting and by the bowel. The high temperature, perspiration and increased respiration increases this water-loss by the skin and lungs. There is therefore a great dehydration of the tissues and the blood volume would be found much decreased with its viscosity increased due to the increased percentage of protein and other solids. This would cause a diminished bloodflow chiefly to the extremities and the surface but also to internal organs, including the overworked heart. There would be an accumulation of acid products due to the poor circulation. Little or no food is taken, while the need for food is above the average due to the high metabolism. Should any food reach the intestine it passes through before absorption can take place. There is an undue loss of the alkali reserve of bicarbonate by bowel. With no nutrition, at first the body storehouses of glycogen and fat are used. When this is exhausted the nutrition is drawn from the skeletal muscles. The vital organs such as the heart and brain are the very last to be called upon. The glycogen is very soon used up; and the fats without the carbohydrates, burn poorly, producing acetone bodies which render the blood even a poorer carrier of CO_2 and other waste acid products of muscle metabolism. As the etiological organism is in dispute we have no specific therapy, but we know that the disease is self-limited. The indications for correction of the disturbed physiology are very evident, first water and next food, supplying the deficient salts as well as nourishment.

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[1] Blumgart and Yens: Jour. Clin. Invest., 1927, **4**, 1

WATER METABOLISM.

All experiments show that withholding water will produce death more rapidly than the withholding of food. A generous water intake increases the total output of nitrogenous waste products.

Water metabolism is of front rank importance in maintaining health and in the treatment of disease and its benefits can be obtained by every medical practitioner without special equipment. He should order the amount of the fluid intake and see that the administration is carried out. This can be checked up by measuring the urine output which in twenty-four hours is roughly 200 to 400 cc. less than the fluid intake except in active perspiration, vomiting (the amount of which should be measured) edema, and diarrhea. With the exception of heart failure and chronic nephritis with edema every adult patient should drink at least a quart (40 ounces) of fluid daily. Urologists regard a copious urine output of prime importance in urogenital infection and usually give a gallon over the twenty-four-hour period. This consists in about 20 glasses per day, 1 every half hour for ten hours. In acute infections of unknown origin an active elimination is one of the most valuable factors in the management of a patient. Underhill has shown that in the anhydremia which results from simple water deprivation simple administration of water rapidly corrects the deficiency. In the anhydremia which results from burns or serious disease, restoration is more difficult because the dilute plasma passes through the damaged capillaries into the tissues. It is necessary to keep up the water treatment until the capillaries recover their normal function.

It is in preoperative and postoperative care of patients that water metabolism reveals its merit most convincingly and it is in such cases that it is most frequently neglected. Dehydration interferes with the patient's whole metabolism and upsets the ratio of the salts to each other. It is most apt to occur immediately after a long general anesthetic where there has been marked perspiration and postoperative vomiting. In such a patient, an accurate record of the amount vomited and the urine output should be recorded. If the vomiting be not troublesome a positive balance can usually be established by giving the patient spoonful doses of water every minute or two while he lies on his right side to insure rapid passage through the stomach to the intestine.

Water is best given by mouth. Fruit juice is an excellent medium as it is agreeable to the patient and supplies sugars and basic salts when the acid radical is oxidized leaving the inorganic base. When it is necessary to inject fluid, water should be given rather than saline or bicarbonate unless one can determine whether acidosis or alkalosis is present. 0.9 per cent sodium chloride would increase an already existing acidosis. Bicarbonate solutions are contra-indicated if there has been sufficient vomiting to produce alkalosis.

If the patient cannot take fluids by mouth water should be given per rectum under about 6 inches pressure into the bowel or subcutaneously into the anterior axillary folds of the pectoral muscles.

In health, water metabolism is as important as in disease although not so apparent. For the average sized adult the usual rule is 6 glasses per day, 1 with each meal and 1 between meals. The solid food averages over 25 per cent of water and roughly makes up the amount lost by the lungs, the skin and the bowel, leaving the urine output approximately equal to the water intake or over a liter daily. When there is no attention paid to the water intake it is remarkable how many normal individuals have a twenty-four-hour urine output of 500 to 600 cc.

Infants and children do not bear water starvation as well as adults. In health they require relatively more than an adult and they do not always obtain all they require. They should obtain 3 ounces of fluid for each pound of body weight until the water intake reaches 40 ounces which is equivalent to the average water intake of an adult. In illness where there is rapid loss of water through perspiration, vomiting or diarrhea, twice the usual amount may be required. The infant or child should be weighed several times daily and water given either by mouth or hypodermically to keep the weight-loss at a minimum.

CHAPTER III.

BIOLOGICAL REACTIONS ON THE BLOOD.

DETERMINATION OF THE BLEEDING TIME.

Apparatus.—Automatic lancet, filter or blotting paper, alcohol and absorbent cotton.

The Test.—Clean the lobe of the ear with alcohol and absorbent and allow it to dry. Hold it firmly to the lower lobe of the ear, applying counter pressure behind the lobe and release the lancet blade. On infants it is best to use the side of the heel.

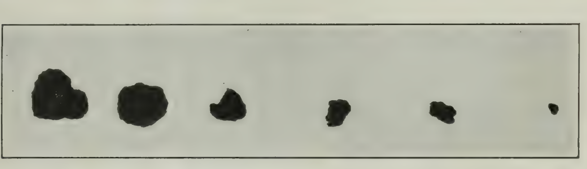

FIG. 38.—Bleeding time following a lancet incision $\frac{1}{8}$ inch deep. The blood was blotted up on absorbent paper every half minute. Bleeding time three minutes.

At half-minute intervals blot up the blood with blotting paper, taking care *not to touch* the incision with the paper. Each blot represents half a minute's flow of blood and the decrease in the size of the drops is an index to the decrease in hemorrhage. The cut should be of such size that the first blot is $\frac{1}{2}$ inch in diameter. The total duration of the hemorrhage is called the bleeding time. Normal two to five minutes.

DETERMINATION OF THE COAGULATION TIME.

The Best Method.

Apparatus.—Capillary glass tube 0.1 mm. to 1.5 mm. in diameter and 10 cm. (4 inches) long.[1]

Lancet, absorbent and alcohol, vaseline.

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[1] Capillary glass tubes may be made by heating any glass tubing until it is soft in a gas or alcohol flame and immediately after removing from the flame drawing the tube out until it is slightly larger than the lead of a lead pencil.

The Test.—Clean the lobe of the ear with alcohol; then coat it with vaseline. Make a puncture $\frac{1}{8}$ inch or more with the lancet, so that the blood will flow without pressure of surrounding tissues. Wipe away the first 2 drops and allow the third to flow into the capillary tube. Hold this tube horizontally in the palm of the closed hand to keep it near body temperature. After three minutes break off $\frac{1}{4}$ inch of the capillary tube and notice if a thread of fibrin bridges the gap as the fragment comes away. If the fibrin thread is not present break off pieces of the tube at minute intervals until it appears.

Normal time two to eight minutes.

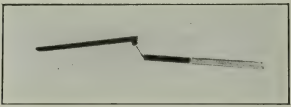

Fig. 39.—Coagulation time. Capillary tube method. On breaking the tube five minutes after filling it, a thread of fibrin bridged the gap.

Interpretation.—The bleeding time is prolonged in hemorrhage of the new-born, thrombopenic purpura and in advanced acute leukemias. The coagulation time is more frequently prolonged in hemorrhagic disease of the new-born, than is the bleeding time. The coagulation time is only slightly prolonged in thrombopenic purpura in contrast to the prolonged bleeding time. In hemophilia the coagulation time is over thirty minutes while the bleeding time from a small incision is normal because the tissue juice from the margin of the incision supplies the blood deficiencies enabling a clot to form.

In many undetermined bleeding states both bleeding and coagulation times are delayed. Apart from hemorrhagic disease of the new-born, examination of the red and white blood cells and platelets give more specific information than the estimation of the bleeding and coagulation time.

A Simple Method.—After puncturing the lobe of the ear through a vaselined spot wipe away 2 drops and let the third remain. After three minutes pass through the drop the tip of a sewing needle that has been coated with vaseline. Fibrin first forms on the surface of the drop and if present will adhere to the needle point. Repeat the test at two-minute intervals.

Comparison of Tests.—The results of all methods are approximately the same. If a small amount of blood is used and it comes in contact with much rough foreign substance it will clot more readily than when larger amounts are used which come in contact only with smooth surfaces. The lower the temperature the slower the clotting. Some authorities maintain that coagulation time should be tested only on venous blood and not on the capillary blood from a skin puncture where the tissue juices may mix with it and so hasten clotting. They recommend the technique of Lee and White in which 1 cc. of blood is drawn from a vein into a syringe that has been rinsed with normal saline. The blood is transferred into a small test-tube which has been rinsed with normal saline and has a diameter of about $\frac{1}{3}$ inch. Tip the tube every half minute enough to move the surface of the blood. When the blood remains solid, coagulation has taken place. It has been stated that this method shows the true coagulation time of the blood but the testing of the second or third drop obtained from a small incision is nearer what actually happens in an injury.

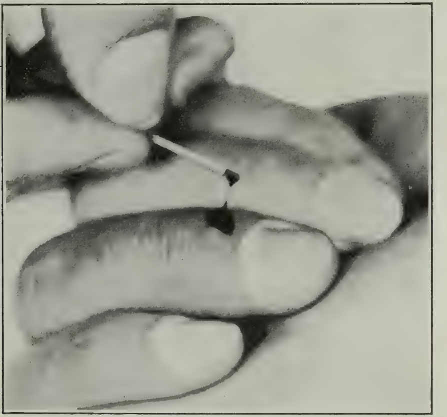

FIG. 40.—Coagulation time. Needle method. The fibrin forms in the top of the drop and can be lifted up in a thread.

THE HESS CAPILLARY TEST.

Apparatus.—Sphygmomanometer and stethoscope.

The Test.—Place the cuff of the sphygmomanometer above the elbow and inflate it until the pulse disappears at the wrist. Lower it 10 points and maintain it at this level for five minutes.

Result.—The test is positive if petechial hemorrhages or blotches appear under the skin. No capillary hemorrhages occur in normal people when the return flow is obstructed in this manner. A positive test denotes weakened and imperfect capillary walls. It is present in widely different diseases such as scurvy, thrombopenic purpura, subacute bacterial endocarditis and acute leukemia.

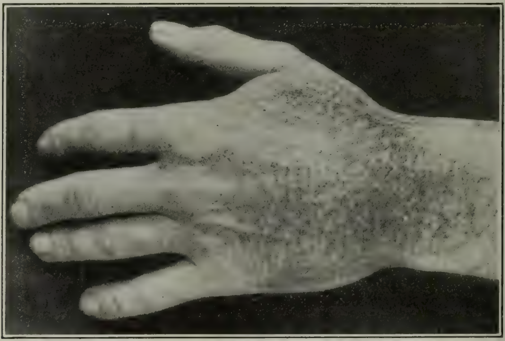

FIG. 41.—Hess capillary test. Positive reaction. Petechæ on the hand after the tourniquet was applied above the elbow five minutes. The patient suffered from monocytic leukemia and died a week later. All the organs were stuffed with leukemic cells.

CONTRACTION OF CLOT.

Apparatus.—A blood vacuum tube or a simple 20-gauge needle and clean dry test-tube, tourniquet, alcohol and absorbent.

The Test.—Obtain from a vein in the arm, enough blood to make a column of 2 or 3 inches in a test-tube. Place the test-tube in a tumbler or rack and note the degree of contraction that takes place at room temperature in the clot one hour and two hours after the blood is withdrawn.

Normal.—The clot contracts leaving a slight margin of clear serum in one hour and definitely in two hours. Retraction is complete in twenty-four hours.

Interpretation.—The rapidity and degree of contraction indicates the character of the clot. Weak friable clots that do not contract will not arrest hemorrhage. The clot contraction runs parallel to the platelet count. No contraction will occur if the platelets are below 50,000 and it will be much delayed when the platelets are below 100,000 per cu.mm. Very few platelets are necessary for clot formation but the number must approach normal for good clot contraction so in thrombopenic purpura there is normal clotting time but delayed contraction of the clot. In hemophilia the increased resistance of the platelets interferes with coagulation but once the platelets are ruptured there are sufficient to cause normal clot contraction. I have seen a woman suffering from spontaneous hemorrhages who had normal platelet count and no contraction of the clot after twenty-four hours. In a hemorrhage from unknown cause a lack of clot contraction indicates a blood defect which may be remedied for a week or more by blood transfusion. This will bridge over the possibility of hemorrhage during and after a surgical operation.

ABNORMAL HEMORRHAGE.

The cause of excessive hemorrhage is not well understood and its management is frequently faulty. The following discussion is an attempt to sift out valuable points in diagnosis and therapy. It deals with the unduly prolonged hemorrhage following injury or local disease where the usual measures fail because there is some deficiency in the clotting power of the blood. Hemorrhage also occurs as a complication of some severe general diseases. Here the cause is weakness of the vessel wall as distinct from the true hemorrhagic disease where the blood is unable to form a firm clot, usually due to defects in the platelets alone.

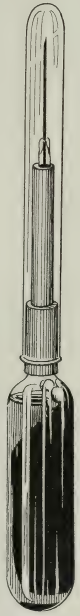

FIG. 42.—Normal clot contraction after two hours at room temperature.

If the bleeding tendency is not marked, hemorrhages occur only when local injury or disease opens the blood-vessels and the blood lacks the proper coagulating powers to plug the opening and continues to drain away.

Any classification of hemorrhage is imperfect but this attempt may be helpful.

GROUP 1. HEMORRHAGES DUE TO INJURIES OR LOCALIZED DISEASE.

Trauma and bleeding after surgical operations.
Epistaxis resulting from an injury or an ulcer of the septum.
Apoplexy.
Esophageal varix, peptic ulcer.
Placenta previa.
Postpartum bleeding.
Ectopic pregnancy.
Uterine hemorrhage due to new growth.
Hemorrhoids and bleeding from any ulcerating tumor.

GROUP 2. SPONTANEOUS HEMORRHAGE AS A COMPLICATION OF A GENERAL DISEASE.—Acute infectious diseases such as pyemia, septicemia, malignant diphtheria, measles, smallpox; also in jaundice, pernicious and aplastic anemias, leukemias, severe malnutrition as scurvy and symptomatic purpuras.

GROUP 3. THE TRUE HEMORRHAGIC DISEASES.—Hemorrhagic disease of the new-born, purpura hemorrhagica of Werlhof or thrombocytopenic purpura, hemophilia, pseudohemophilia.

Howell's Theory of Blood Coagulation.—There are in the circulating blood, fibrinogen, prothrombin and antithrombin. The antithrombin inactivates the prothrombin. When blood is shed the platelets rupture and liberate thromboplastin which combines with antithrombin and so liberates prothrombin. The prothrombin, activated by the calcium unites with the fibrinogen to form fibrin. The estimation of these substances cannot be applied in a practical way to hemorrhage but the whole problem of blood clotting is very much associated with blood platelets. (See page 74.)

The following is a classification of the hemorrhagic states on the basis of anatomical origin.

1. Chemical disturbance of the blood such as occurs in severe jaundice.

2. Endothelial weakness or damage either by lack of nutrition as in scurvy or in the toxemia of severe septic infections.

3. Diminution or defect in platelets. The platelets are diminished in true purpura and unduly resistant in hemophilia.

4. General diminution of all myeloid elements (myelophthisis). Here the red cells, white cells, especially the leucocytes, and the platelets are all diminished as in aplastic anemia or extensive carcinomatous involvement of the bone-marrow. The underlying disease is usually severe and the prognosis a fatal one.

More than one of these causes may be present.

Group 1. Hemorrhages Due to Injury or Local Disease.—*Procedure in Diagnosis.*—If the hemorrhage persists after all local measures have been applied, such as vessel ligation, hot compresses and coaptation of surfaces, especially of muscle, there is usually faulty clotting of the blood. *It is well to reëxamine carefully for open bloodvessels however.* The formation of a clot at a bleeding point does not mean that the blood has normal hemostatic powers. The clot may be of poor quality. The clot contraction capacity as demonstrated in a test-tube is a more reliable indicator. This takes an hour or two so that the time required limits its usefulness. The test for bleeding time and coagulation time are easily done but are not always reliable indicators. A blood smear for examination of the platelets, red and white cells and, when facilities permit, *a count of each,* will often give valuable information in excluding thrombopenic states, severe anemias or leukemias.

Only 2 out of 3 of the patients who have oozing of blood from a wound after ordinary hemostasis will show any abnormality of the tests supposed to demonstrate faulty coagulation. The bleeding time, the platelet count, calcium content and other chemical constituents may be normal in a patient who bleeds persistently from a surgical wound. Nevertheless some defect usually exists and goes undetected only because our present investigation is inadequate.

Acute Blood Loss.—The maximum blood-loss which may occur without causing death depends on the rapidity of the hemorrhage. The total quantity of blood is about 8 per cent of body weight and if a massive hemorrhage occurs rapidly the patient will not survive a loss of more than one-third the total volume. Should the hemorrhage extend over twenty-four hours two-thirds of the total blood volume may be lost before causing death. Women have a greater tolerance to blood-loss than men. Old people and children bear hemorrhage very badly.

Blood Changes.—For two hours after a hemorrhage no change occurs in cell counts or hemoglobin. A block of the blood volume is lost and the vascular system by contracting adapts itself to the lessened amount. The pulse is small and irregular during this period if the hemorrhage is great. A falling blood-pressure is therefore a more reliable sign of continuing hemorrhage than blood cell counts. About two hours after a hemorrhage, blood counts and hemoglobin estimation will by their decrease, detect a beginning increase in the blood volume. At first there is absorption of salts and water from the tissues, later it comes from the intestine. The intense thirst shows the need of fluid.

The diminution of hemoglobin, red cells and blood protein does not reach its maximum for two weeks at which time the patient feels well on the road to recovery.. If the hemoglobin goes below 25 per cent death frequently occurs. The blood volume which increases rapidly during this time remains slightly subnormal as long as anemia persists.

Four hours after a hemorrhage occurs there is an increase in the leucocytes which increase may persist for a week. In twenty-four hours the platelets begin to mount and in forty-eight hours after a severe hemorrhage there may be two to three times their normal number. During the first twenty-four hours there is a drain on normal red cells in the bone-marrow reservoirs but after this time young forms having reticulation with vital stains (reticulocytes) appear and in a week may reach 10 to 15 per cent. Many large hemoglobin-poor, ghost-forms, a few polychromatophilic cells and some normoblasts may also be seen. The cell increase is more rapid than the hemoglobin increase which accounts for the color index being below *one* in hemorrhagic anemia.

After a single hemorrhage of a quart the blood comes back to normal in six weeks. After more severe prolonged or repeated hemorrhages three to six months may elapse before complete recovery takes place even when the bone-marrow has normal regenerative vigor. When it is exhausted recovery may not take place after twelve months.

In the order of their importance, treatment should be directed to (1) arrest the hemorrhage by surgical or hemostatic means, (2) restoration of the blood volume by adequate fluid intake or better by a transfusion which will not only restore the blood volume but also the plasma protein and cells.

Transfusion is rarely necessary when the hemorrhage is less than a quart or the hemoglobin below 60 per cent.

General Principles in Treatment.—Two simple but valuable fundamentals should not be overlooked. (1) A retarded circulation gives the clotting process a better chance to act. Thrombosis is usually associated with blood stasis and if the heart action be slowed down by some powerful sedative like morphine the blood has a much better chance to clot. (2) General and local warmth aid in the clotting process. Blood clotting is a chemical action and it has been clearly proven that temperatures slightly higher than that of the body hasten the process. When a specific bleeding disease has been diagnosed special management for that disease as well as local treatment should be used.

Local Treatment of Accessible Hemorrhage.—Sometimes compression, hot sponges, and vessel ligation fails to stop a hemorrhage. After tonsillectomy a common cause of bleeding is a rupture in the wall of a vein and having the patient sit up will stop it.

When standard methods of hemostasis fail a gauze sponge wrung out of adrenalin chloride 1 in 10,000 is the next step. After wiping away any bloodclot apply 3 or 4 of the sponges each for several minutes and leave the last *in situ.* If the bleeding does not stop or if it recurs apply sponges moistened with cephalin. Of all the remedies applied as local styptics the most efficacious is cephalin or brain extract.

Cephalins are the only hemostatic substances mentioned in "New and Non-official Remedies," by the Council of Pharmacy and Chemistry of the American Medical Association, 1929. There are a host of others in common use but critical experiment has found them inadequate. Calcium, which has long been recommended, is only of value in a few cases which have a lack of calcium in the blood. Cephalin is soluble in ether and forms a suspension in 0.9 per cent of sodium chloride. In solution or solid form it gradually loses its power to aid the blood in clotting so that Armour's and Lederle's product have an expiration date on the bottle label after which the contents should not be used. Some manufacturers market brain extract as thromboplastin.

Management of Inaccessible Hemorrhage.—When the source of bleeding cannot be easily reached or when the above-outlined treatment fails one must resort to general subcutaneous injections. The action is much more definite if the injection be made into or around the bleeding area. Cephalin which has been specially prepared for subcutaneous or intramuscular injection is the first choice.

When these hemostatics are not available, fresh whole blood from a normal individual is the next choice. It may be injected intramuscularly without preliminary grouping and in 20 to 50 cc. amounts has a much greater hemostatic action than a similar amount of normal horse serum, antidiphtheritic serum or the usual run of hemostatic substances on the market. It is not so efficacious as the intravenous transfusion.

Diet has effect on bleeding tendencies. Lipins and globulins, which are contained in liver, kidney, brain, caviar, egg-yolk, bacon, nuts, beans and peas produce a definite increase of blood clotting powers within twenty-four hours.[1] A diet consisting of vegetables low in food value such as cabbage, tomatoes, cauliflower and stewed fruit will decrease the clotting function of the blood.

Transfusion of normal blood is without doubt the most reliable method of supplying any deficient substance without which the blood fails to clot properly but a knowledge of the blood groups, which may be easily obtained, is necessary before transfusion is safe.

Gastric hemorrhage deserves special mention. If the patient has lost much blood he will not survive an immediate surgical operation. The best choice of immediate treatment is rest and morphia to lessen the movement of the stomach. Sucking ice or chewing gum to stimulate the salivary flow and small but frequent doses of alkalies to neutralize the gastric acidity.

Hunter[2] states that hydrochloric acid weakens the bloodclot, making it jelly-like while saliva hastens the clotting process in the same manner as tissue fibrinogen.

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[1] Kugelmass *et al.*: Am. Jour. Dis. Child., 1930, **39**, 471.

[2] Brit. Jour. Surg., 1928, **16**, 203.

Most of the evidence favors a small transfusion of 200 to 500 cc., repeated if necessary. This relieves the collapse, the anemia and any blood clotting deficiency which may be present. A hemoglobin below 60 per cent is a definite indication for transfusion. Larger transfusions by suddenly raising the blood-pressure tend to displace the thrombus and so start the bleeding again where the facilities for transfusion are not available and the shock and collapse are severe and it may be necessary to give a slow infusion of 1 or 2 quarts of sterile water into the anterior folds of the breast. When the patient has recovered from the immediate effects of the hemorrhage, surgical exploration should be considered.

Group 2. Hemorrhages Occurring as a Complication of General Disease.—*Infectious Fevers.*—Hemorrhages are most frequent in the very severe forms of infectious fevers. The lesion is usually capillary wall defect. An excess of antithrombin has also been demonstrated. The hemorrhages do not form a danger factor in the disease.

The petechiæ and hemorrhagic nephritis of subacute endocarditis are not always due to emboli. There is weakening of the endothelium as shown by the frequently positive Hess capillary test. The same is true of many of the petechiæ occurring in generalized septic diseases.

Jaundice.—Jaundice that causes hemorrhage is a cholemia resulting from obstruction in the biliary ducts, or toxic hepatitis, which dams back *bile salts* as well as bile pigments into the blood stream. Bile salts of 0.4 per cent concentration will dissolve a clot already formed. Calcium chloride 10 cc. of a 5 per cent solution injected intravenously will arrest the hemorrhagic tendency in most cases for three or four days. The coagulation and bleeding time are not as reliable indicators of the hemorrhagic tendency in jaundice as is the blood cell sedimentation rate. When it remains accelerated after the injection of calcium chloride a blood transfusion should be given because extensive damage to the liver cells may reduce the fibrinogen content of the blood and a transfusion remedies this as well as other deranged elements.

Anemia.—The platelets are increased in secondary anemia and decreased in the pernicious or aplastic types so that spontaneous hemorrhage may result. In aplastic anemia there is a diminution of all the cell elements in the blood, red cells, white cells, especially the polymorphonuclears and the platelets. The prognosis, except in benzol poisoning and after *x*-ray exposure is grave.

In the lymphatic type of leukemia the lymphoblasts invade the bone-marrow and crowd out the megakaryocytes from which the platelets develop; so that the blood platelet count is usually low. In myelogenous leukemia the platelets are usually enormously increased because of the general bone-marrow proliferation but they sometimes may be decreased. Spontaneous hemorrhage may occur in leukemias with a decrease or an increase of the platelets. The Hess capillary test is positive suggesting that weakness of the capillary wall plays an important rôle in the hemorrhage. The patient who has a greatly diminished leucocyte count, necrotic gums, ulcerative stomatitis with hemorrhages has aleukemia hemorrhagica. Blood transfusion rarely helps even temporarily.

Scurvy.—While in scurvy the site of the hemorrhage is usually from mucous membranes, when it is in the skin it is confined to the hair follicles. The hemorrhagic spots are therefore small and numerous unless the condition be severe and many coalesce. The surrounding small foci distinguish it from thrombopenic purpura where the hemorrhages are large and not confined to the hair follicles. In scurvy there is a history of faulty diet and the gums are spongy, ulcerated and bleed readily. The blood platelets are normal and there is usually a secondary anemia after the condition has existed some time. A swelling and loosening of the intercellular cement substance has been demonstrated histologically. This is the cause of the rupture of the vessels with hemorrhages into the tissue. The Hess capillary test is positive.

Symptomatic Purpuras.—These are the purpuras which are due to capillary wall defect and in which the platelets are normal. In contrast to the true purpura in which the skin lesion is purely hemorrhagic, symptomatic purpura is usually accompanied by urticarial itching. The skin appearances are pleomorphic and as Christian states, there are as many names as there are skin appearances. The purpura simplex, Schönlein's, which is purely subcutaneous, Henoch's which is more severe with abdominal lesions, all the erythemias, the urticarias and angioneurotic edema result from various combinations of serum, red cells or white cells which may penetrate the vessel wall producing the skin lesion. Similar lesions, may occur in the mesentery and bowel, producing colicky pain. The hemorrhagic tendency may also be the cause of arthritis, hematuria or menorrhagia. The clotting is normal. The bleeding time may be normal or slightly lengthened. The blood platelets are normal. There is a slight leucocytosis due to infections and after the state has existed for some time there may be anemia. Hemorrhages when they occur are not dangerous. They are usually into the tissues and from the gums. Treatment should be directed to the primary cause.

Group 3. The True Hemorrhagic Diseases.—*Hemorrhagic Disease of the New-born.*—Hemorrhage of the new-born is a tendency to bleed from small vessels in the gastro-intestinal tract, in the umbilicus or into the subcutaneous tissues. There may also be gross interval hemorrhages. During the first week in the life of a normal infant the bleeding time may be normally prolonged to five minutes and the coagulation time to ten minutes. Hemorrhages occur in most cases where they are more prolonged. The coagulation and bleeding times are therefore important and should be performed if any unusual symptoms present themselves or routinely on the first and fourth days. These tests serve to distinguish hemorrhagic disease from hemorrhage wholly due to birth trauma of forceps or breech delivery, asphyxia, prematurity, toxemia in the mother, gastro-intestinal ulcers, acute infections or syphilis.

Cerebral hemorrhage which is very common may result from birth trauma in difficult deliveries, or a hemorrhagic tendency or it may result from a combination of these two. A bulging fontanelle or any cerebral symptoms in a new-born baby who cries persistently warrants a diagnostic lumbar puncture, and tests to determine any hemorrhagic tendency.

In hemorrhage of the new-born there is frequently a lack of prothrombin. Hemorrhage is more frequent in infants whose mothers, because of toxemia of pregnancy, were fed over a long period on a diet deficient in water soluble vitamin B (vegetables and cereals).[1] If 20 to 50 cc. of whole unmatched blood be given subcutaneously the bleeding will stop in most cases in an hour or two. Where this fails or where there has been much blood-loss, transfusion of properly matched blood is indicated.

True Purpura, Thrombopenic Purpura of Werlhof.—This hemorrhagic state which is usually intermittent is associated with a decrease of blood platelets, below 100,000 per cu.mm. and is most likely due to an excessive phagocytosis of the platelets by the spleen. The bleeding time is always prolonged although the clotting time may be almost normal. If a sample of blood be placed in a test-tube there is no retraction of the clot. If much bleeding has occurred anemia will be present and it may be confused with aplastic anemia where the platelets are also low. Aplastic anemia may be excluded if there is a high percentage of reticulated red cells and a normal percentage of neutrophile leucocytes even when the total white count was not high.

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[1] Moore and Brodie: The Relation of Maternal Diet to Hemorrhage of the New-born, Am. Jour. Dis. Child., 1927, **34**, 53.

The hemorrhages are usually from the mucous membranes and bowel. It may be acute or chronic, mild or dangerous and there is frequently splenic enlargement. As a treatment blood transfusion will give relief for a week or two. *X*-radiation of the spleen produces more permanent relief and for refractory cases removal of the spleen is justified. The platelet count rises very rapidly for a few days following splenectomy but after several weeks it may decrease to about 100,000 per cu. mm. but the patient will have few or no symptoms.

Hemophilia.—This is a rare familial disease found only in males and transmitted through females. There are periodic attacks and a fatal hemorrhage may occur from a small wound. The blood coagulation time is very much lengthened. The bleeding time from a small puncture wound may be normal, due to the admixture of tissue juices. The blood platelet count is normal but the platelets do not agglutinate and are unduly resistant and so do not liberate the thromboplastin at a bleeding surface. All other components of hemophilic blood have been found normal. As treatment, blood transfusion offers the most dependable and immediate relief. The improvement lasts only a week however. In an emergency the patient's own blood, either citrated or defibrinated by shaking with glass beads, may be used for transfusion. Sodium citrate given intravenously has some beneficial effect. Christie and Gulland have found serum and tissue extracts of no benefit. They recommend that a pledget of cotton wool soaked in fresh human blood should be applied locally to a bleeding lesion after the useless blood clots have been wiped away.

Pseudo-hemophilia of Hereditary Diathesis.—This disease occurs most frequently in asthenic individuals and is inherited by men and women alike. There is usually a normal bleeding and coagulation time and the platelet count is usually normal. At times there may be a temporary thrombopenia and a lack of clot contraction powers. The remedies advised for hemophilia help.

THE WIDAL AGGLUTINATION TEST.

Simple Bedside Method.

Apparatus.—Lancet, glass slides, alcohol and absorbent, typhoid culture formalinized.[1]

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[1] To an eighteen- to twenty-four-hour culture add enough formalin to make it 0.25 per cent strength of the total culture. This kills the organisms but does not destroy their agglutinating properties. The solution will keep in an ice-chest for several months but must be always thoroughly shaken before use to destroy any spontaneous agglutination which may occur.

Summary of Hemorrhagic Diseases.

	Athrombopenic purpura.	Hemorrhage of the new-born.	Thrombopenic purpura.	Hemorrhage of anemia and leukemia.	Hemophilia.	Pseudo-hemophilia.
Lesion	Weakness of capillary walls	?	Deficiency of blood platelets	Deficiency of cells and platelets	Failure of blood to clot	?
Etiology	*Infections*, allergic states, serum sickness, deficiency of vitamin C in foods in scurvy	Sometimes associated with toxemia of pregnancy, prematurity of difficult labor	Abnormal phagocytosis of the platelets by endothelial cells in spleen	Crowding out of parent cell in the bone marrow, exhausted states of the bone marrow	Unduly resistant platelets which do not easily rupture to form fibrin; an hereditary bleeding tendency in males	Sometimes hereditary affects females as well as males
1. Clotting time	Normal or *shortened*	*Prolonged*	Normal	Normal	*Greatly prolonged*	*Irregular*, frequently lengthened temporarily
2. Bleeding time	*Prolonged*	*Prolonged*	*Prolonged*	Normal (small incision)	Normal (small incision)	Normal
3. Clot contraction time	Normal	Normal	*Prolonged*	Normal	Normal	Prolonged at times
4. Platelets	Normal (*approximately*)	Normal	Lessened or *absent*	Lessened in *aplastic anemia*	Normal in number but do not break up with usual readiness	Diminished at times
5. Red cells	Normal	Normal	Normal	Lessened in anemias	Normal	
6. White cells	Slight (due to infection)	Normal	In acute form infectious leucocytosis	Usually an increase in leucocytes, always an abnormal differential count	Normal or slight lymphocytosis	
Clinical	Pleomorphic exanthem; bleeding from gums; hemorrhage into tissues; usually a benign course, seldom fatal; in scurvy subcutaneous hemorrhage hair follicles; capillary test positive	Frequent on third day; may occur first five days; hemorrhage may come from bowels, lungs, urethra or be intra-cranial, producing convulsions and a blood-stained C.S.F.	Extensive subcutaneous hemorrhages, frequently from mucous membrane; acute or chronic, benign or severe; sometimes splenic enlargement; capillary test positive	Weakness, yellowish pallor; hemorrhages from mucous membranes; may occur in aleukemic leukemia of the acute type; capillary test positive	Prolonged bleeding from small wound or into joint, periodically worse and sometimes fatal; capillary test negative	Resembles hemophilia in many respects but bleeding is not so severe
Therapy	Search and treat the cause if found; meanwhile treat symptoms	20 to 50 cc. of normal whole blood subcutaneously or transfusion of 100 cc.	*X*-ray of spleen or splenectomy	Liver diet for anemia; blood transfusion; *x*-ray for leukemia	Blood transfusion; sodium citrate intravenously	As for hemophilia

The Test.—Spread a small drop of the patient's blood on a glass slide as for a cell examination. After it has dried add 4 drops of distilled or tap-water and tilt the slide from side to side so as to hemolyze all the cells. Now add 4 drops of the formalinized culture of typhoid bacilli and again tilt the slide to mix the solutions. If the reaction be positive, agglutination, appearing as a fine curdy flocculation, will occur in one minute. If in doubt about the result drain off the excess fluid on the slide, dry and fix over a flame, stain with methylene blue and examine under the low-power lens of the microscope. When the test is positive the bacilli are seen in huge clumps. A control test done on normal blood shows the bacilli quite evenly distributed. This reaction will occur on blood that has remained on a slide for six months.

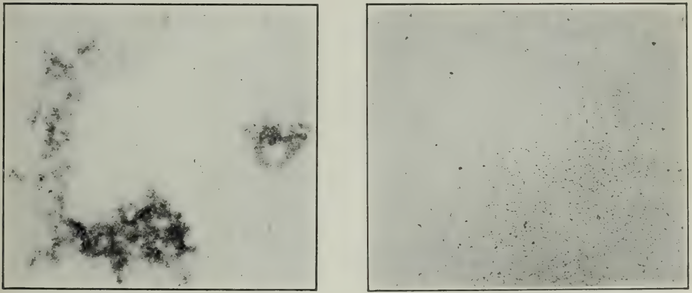

Fig. 43.—Widal positive.

Fig. 44.—Widal negative.

Figs. 43 and 44.—Microscopic appearance under the low-power lens (× 100). The mixture of hemolized blood and formalinized typhoid culture has been drained off the slide and the remaining bacteria stained with methylene blue.

Microscopic Quantitative Method.—Fill a glass capsule not more than two-thirds full of blood and seal the end that contains no blood over a flame. Centrifuge it with the sealed end to the outside. File the capsule just above the serum and break off the open end. Draw the serum into a leucocyte pipet up to mark 0.5 and dilute with normal saline to 11. Mix 1 drop of this diluted serum with 1 drop of typhoid culture on a cover-slip and place over a hollow-ground slide that has been ringed with vaseline. Clumping will take place in five minutes at room temperature if the reaction is very positive. After thirty minutes and after one hour examine under the high power of the microscope for loss of motility and for clumping. The clumping should be very definite before reporting the reaction positive. A drop of the culture to which has been added a drop of saline instead of the diluted serum, should be examined to rule out any spontaneous clumping that may take place in the culture. The stem of the leucocyte pipet is divided into 10 equal parts and by filling it to these various marks and diluting with saline, agglutinative tests may be set up varying from 1 in 20 to 1 in 200. Set up similar tests to paratyphoid A and B. If 1 in 40 is positive another should be done at 1 in 100 and 1 in 200.

Interpretation.—A definitely positive agglutination reaction with a serum dilution of 1 in 40 may be taken as evidence of typhoid infection provided the patient has not been inoculated during the previous year. The reaction occurs in about one-half the patients who have had typhoid infection and fever for a week. The percentage of positive reactions gradually increases up to the fourth week when almost 100 per cent show a positive reaction, even with serum diluted up to 200. Some months after the patient recovers the agglutination reaction becomes negative. If it remains positive over a year the patient probably has a gall bladder infected with typhoid bacilli and is a carrier.

It is important to know the dilution at which agglutination occurs. A positive reaction at a dilution of 1 in 20 while highly suggestive of typhoid may also occur in miliary tuberculosis. If complete agglutination takes place in five minutes with a dilution at which the reaction occurs, the more certain is the diagnosis typhoid.

BLOOD CULTURE.

PRINCIPLE.—Very few microörganisms in blood obtained from a vein under aseptic precautions, if cultured at 98° F. for several days will show a profuse growth of organisms that could not be detected by ordinary examination.

Apparatus.—Culture media. (For occasional use the large vacuum tube containing glucose broth is best. It is made by Hynson, Westcott & Dunning, Baltimore, Maryland. If this be not available, collect the blood with a sterile syringe. Immediately place it in a sterile tube containing citrate or oxalate to prevent clotting. In this way it may be transported to the laboratory where it is deposited in glucose broth. The culture tube should contain media 6 inches in depth. A cube of brain tissue in the media enhances the growth. It is better if the syringe full of blood can be immediately inoculated into the media. Convenient blood culture tubes may be made by having a large test-tube stoppered with a cork containing two holes which contain glass tubes, one connected by rubber to a 20-gauge needle and the other stuffed with wool connected with a mouthpiece. The needle is sterilized and covered with a small test-tube. *The risk of contamination increases with the number of manipulations in transferring the blood from the vein to the culture media.* Bile media is best in suspected cases of typhoid.)

Rubber tube, tourniquet.

Alcohol, absorbent, 2 per cent mercurochrome in alcohol-acetone solution or tincture of iodine.

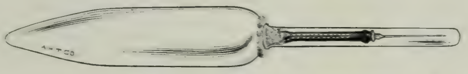

FIG. 45.—Blood culture tube of the vacuum Keidel type containing glucose broth.

Obtaining the Specimen.—Prepare the site of needle puncture with great care. The veins at the bend of the elbow are the most accessible. First wash the skin with a liberal amount of alcohol, using two or three swabs. After it dries paint on mercurochrome or iodine and allow it to dry. Apply the tourniquet above the elbow moderately tightly so as not to occlude the pulse at the wrist. Instruct the patient to open and close the hand several times and finally to keep the fist clenched while the needle is being inserted into the vein. Withdraw 2 to 4 cc. of blood, depending on the amount of media available. To avoid the bacteriostatic action of blood, it is very important that it should be diluted by five to ten times its quantity of media.

Naked-eye Examination of the Culture.—After twenty-four to forty-eight hours of incubation the culture may be examined for microörganisms. If there be a prolific growth the media will be cloudy throughout. This usually occurs in puerperal sepsis. In the deep tubes the opacity may occur at a certain depth due to some streptococci being partially anaërobic. When the organism is a streptococcus viridans as in endocarditis, growth is very slow and it may require a week to develop enough for examination.

Microscopic Examination.—(*a*) *With the Vacuum Tube.*—There is considerable difficulty in obtaining a loopful of the media and sealing the tube. The most satisfactory method is—flame the shoulder of the tube and after filing the neck, break it off and insert a platinum loop with a very long wire which has been sterilized in the flame. Remove the drop from a cloudy portion of the media and place it on a glass slide for microscopic examination. After flaming the shoulder and neck of the culture tube cover it with an inverted small sterile medicine glass rather than trying to plug the small irregular opening with sterile absorbent. In case ordinary culture tubes plugged with absorbent are used they should be held off the perpendicular so no microörganisms from the air will drop into the media. Flame the plug before insertion. The drop of culture on the slide should be fixed in the flame, stained with methylene blue or Gram's stain and examined under the oil-immersion lens of the microscope. If no organisms are present the blood culture should be replaced in the incubator and not discarded until it has remained sterile for ten days from the time it was taken.

If organisms be found a subculture may be necessary to fully classify them. Streptococci should be planted on blood agar to determine whether they are hemolytic or non-hemolytic. If pneumococci be present it may be necessary to type them. If Gram-negative bacilli be present a subculture in neutral red glucose and lactose broth will determine whether the organism be colon, typhoid or paratyphoid.

Interpretation.—Bacteriemia denotes the presence of bacteria in the blood stream whereas septicemia implies invasion of the blood stream by bacteria, producing a severe disease of the body tissues. The finding of staphylococcus alone should be regarded as a contamination except where there is a history of the original injury being on the skin. A large percentage of the fatal infections about the hand are due to the staphylococcus. Septicemia is a serious condition. Of every 3 patients that have it about 2 die, the death-rate being higher in postpartum infections, osteomyelitis, influenza and mixed infections. In acute endocarditis, the hemolytic streptococcus is the commonest etiological organism and in the subacute type, the streptococcus viridans. The prognosis is more favorable in otitis media, streptococcus viridans, pneumococcus and staphylococcus infections. The outlook is especially good in typhoid fever as positive blood cultures may be obtained from 80 per cent of cases during the first week of fever.

SERUMS AND VACCINES OF PROVEN VALUE[1]

Serums: Anaërobic antitoxin (for gas gangrene).
Diphtheria antitoxin.
Erysipelas streptococcus antitoxin.
Scarlet fever streptococcus antitoxin.
Tetanus antitoxin.
Antianthrax serum.
Antimeningococcus serum.
Antipneumococcus serum (Type 1 only).
Antisnake-bite serum.

Vaccines: Antirabic vaccine.
Acne bacillus vaccine.
Cholera vaccine.
Plague bacillus vaccine.
Pneumococcus vaccine for corneal ulcer (pneumococcal).
Smallpox vaccine.
Staphylococcus vaccine.
Tuberculins.
Typhoid vaccine.
Autogenous vaccines.

Schick test to determine immunity to diphtheria.
Dick test to determine immunity to scarlet fever.

To Develop Prolonged Immunity—For Diphtheria—Toxin antitoxin mixture and toxoid.

For Scarlet Fever—Scarlet fever streptococcus toxin and ricinoleated antigen.

THE WASSERMANN REACTION.

PRINCIPLE.—In active syphilitic disease the blood contains antibodies, which react against the infection. In the test-tube these antibodies sometimes called amboceptor, will bind complement in the presence of a special antigen. After this reaction has taken place, the bound complement cannot join with hemolytic serum to hemolize sensitized red cells which are later added. So when syphilitic antibodies are present in the serum used, no hemolysis of the red cells occurs and they sink to the bottom of the tube leaving a clear fluid above. When no syphilitic antibodies are present the complement is free to join with the hemolytic serum and cause

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[1] According to the Council of Pharmacy and Chemistry of the American Medical Association, 1929, as published in New and Non-official Remedies. Every practitioner should have this manual. By it he is advised of recent preparations having a sound therapeutic basis and warned about worthless remedies whose vendors shower him with samples and pamphlets having unwarranted claims.

hemolysis of the sensitized cells which colors the contents of the tube a bright red.

This reaction is sometimes called the complement-fixation test and may be used to diagnose hydatid disease, gonococcus infections, tuberculosis, typhoid or other bacterial diseases, by using a special antigen of the etiological organism. Other diagnostic methods are more practical for these infections however.

Apparatus.—(*a*) For occasional use the vacuum tube either of the Keidel or Venule type is most convenient.

(*b*) Other methods are (1) 10-cc. syringe with 22-gauge needle or (2) 20-gauge needle and test-tube. All apparatus coming in contact with the blood must be scrupulously clean and dry. If the test is not done within twenty-four hours collect the blood in a sterile container. (3) Tourniquet of rubber tubing, alcohol and absorbent cotton.

Obtaining the Specimen.—Although the blood may be taken with the patient seated it is best to have him recline to avoid any chance of faintness. The arm of the patient is exposed and the tourniquet applied above the elbow. If the veins are small they may be much dilated by (1) opening and closing the hand, (2) slapping the vein vigorously to paralyze its walls and allow it to dilate, (3) having the patient swing his arm or (4) immersing it in a hot-water bath. In fat individuals where no vein is visible one may usually be palpated.

Wash the area well with alcohol and absorbent. Allow it to dry. If the patient be unduly sensitive a drop of local anesthetic injected intradermally at the site of the puncture will prevent further pain. This is hardly worth while in using the vacuum tube because its needle is not much larger than the hypo needle.

The Puncture.—With the thumb of the left hand on the left side of the vein to steady it, insert the needle slowly at an angle of 20 degrees to the skin surface on the right of the vein opposite the left thumb. If a vacuum tube is being used, break the glass tip after the needle point has passed under the skin. Push slowly to enter the vein when blood will appear. Hold the needle very still. *Collect* 5 *to* 10 *cc.* Release the tourniquet and pull out the needle. Immediately apply a small gauze pad over the needle wound[1] and to prevent a hematoma from forming, tell the patient to apply firm pressure for several minutes. No dressing is necessary. It should reach the laboratory within twenty-four hours without being subjected to much agitation or extremes of heat or cold.

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[1] If a gauze pad saturated in alcohol be placed over the needle-point of a vacuum tube before it is pulled out, as the needle leaves the skin it will draw in a few drops of alcohol which vitiates the results of the test.

The vacuum tube is best because it is always dry, clean and sterile. Water even in small amounts will cause hemolysis of red cells, chemicals will interfere with the result of the test and when all the apparatus is not sterile bacterial growth will take place after twenty-four hours if the blood be not kept in a refrigerator.

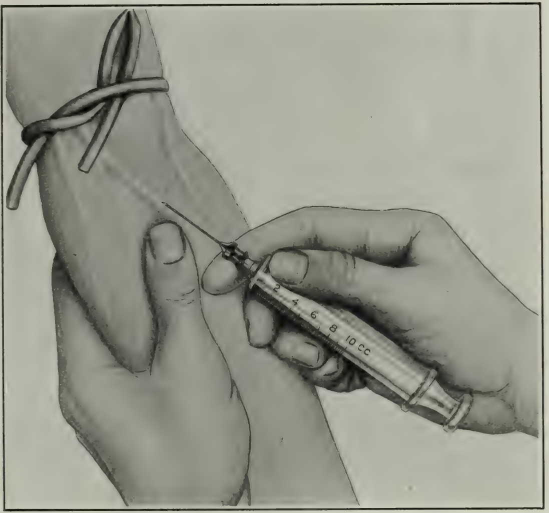

FIG. 46.—Obtaining venous blood. Press the needle through the skin and vein wall slowly. Apply suction on the piston with the left hand. When syringe is filled remove the tourniquet. Withdraw the needle, apply a gauze sponge to the needle puncture and have the patient apply pressure. Remove the needle from the syringe and discharge the blood into the test tubes or bottles.

Five cubic centimeters is ample for a single examination and 10 cc. for several. See special directions for blood culture, serum or chemical examination. For Wassermann icterus index or serum calcium do not use an anticoagulant. For chemical tests use potassium oxalate (sodium fluoride to preserve blood sugar on standing). Hemoglobin, cell counts, cell volume (using correction) and cell settling time may be done on venous blood if an anticoagulant has been added. This obviates the annoyance of multiple punctures.

Other Apparatus.—When a vacuum tube is not available a clean, dry sterile 22-gauge needle 1 inch long and 10-cc. glass syringe may be used. After pressing the needle firmly on the syringe, puncture the vein as already directed and draw gently on the piston until 5 cc. of blood are obtained. Withdraw the needle from the vein, separate it from the syringe and expel the blood into a clean, dry sterile bottle. Some prefer to puncture the vein with a 20-gauge needle and allow the blood to drop into a test-tube. This may be done when the veins are large and prominent but the large needle is painful.

Transporting Sample for Wassermann Test Over Long Distances.—If the blood is to go on more than a two days' train trip to the laboratory it is best to send the serum only, because if whole blood is sent the cells may become hemolized. After the blood has stood three to six hours in a warm room the clot will contract. If the clot is high in the test-tube centrifuging will drive it to the bottom. Pipet off 1 to 2 cc. of *clear* serum and place it in a vial with a new cork stopper. The pipet and vial must be clean, dry and sterile as well as all other apparatus employed in obtaining such a specimen. Every extra step increases the hazard of contamination. After a thorough cleaning with soap and water the containers should be rinsed three times in distilled water. One hour in an ordinary cooking oven will dry and sterilize the glassware and needle.

The Test.—This test is so highly technical that it should be done at a large laboratory where a competent serologist is giving special attention to the reaction and where there are sufficient negative and positive controls to judge the accuracy of the result. The original procedure yielded satisfactory results, but the amounts of material used were too large, so that now small amounts of the reagents are used. The Kolmer modification has the advantage of standardization of the reagents as well as the technique and gives very reliable results. Most laboratories report the result using the method of Citron, namely: Negative, Doubtful, 1, 2, 3, and 4 plus positive.

Antigens.—An extract of syphilitic liver was formerly used as an antigen. Later alcoholic extracts of normal heart were used. The acetone insoluble antigen had many supporters. Most laboratories at the present time use cholesterinized antigens. They are very highly sensitive and when carefully employed with known positive and negative sera give good results.

Sometimes highly cholesterinized antigens give partial positive reactions in pregnancy, but the correct diagnosis may be ascertained by doing repeated tests in several laboratories, by the use of less sensitive antigens or by investigation of the marital partner.

There is an increasing interest in precipitation reactions of which there are many based on the Meinicke reaction. The Kahn modification is very simple and rapid and yields satisfactory results, agreeing with the Wassermann reaction in 90 per cent of cases. It shows a positive reaction earlier than the Wassermann and is not so readily turned negative by treatment. However the consensus of opinion is that in different laboratories using a standard technique results vary more with the Kahn test than with the Wassermann. Recently the United States Naval Medical Corps have adopted the Kahn test instead of the Wassermann procedure.

The Report[1] depends on.

1. *Variations in the Patient.*—If the serum of a syphilitic patient be examined at weekly intervals the reaction will vary in the slight cases between negative and slightly positive, and in the advanced cases between slightly and markedly positive. There are remissions and exacerbations of the disease and the Wassermann test is positive only when the disease is active. A high fever or anesthesia will produce slightly positive reactions. Drinking spirits will occasionally produce a negative reaction for several days in a syphilitic patient.

2. *Collection of the Specimen.*—Many specimens received in laboratories cannot give accurate results because the blood may be hemolyzed, mixed with chemicals or infected with bacteria. Freezing or shaking will cause hemolysis. If the blood stands in a warm room for over forty-eight hours hemolysis will take place. Mere traces of chemicals such as sulphuric acid or phenol which may be present in corks or the containers may contaminate the blood. The labelling is a very important matter. The doctor taking the blood should attach a well-gummed label or adhesive on the container and write the patient's name on it with ink.

Interpretation.—Positive Wassermann reactions occur in untreated definite clinical syphilis as follows:

1. In the chancre stage 30 per cent after one week to 90 per cent after six weeks.

2. In the secondary stage with a rash, mucous patches in the mouth or condylomata, 100 per cent.

3. In tertiary syphilis over 90 per cent show a positive reaction.

4. In congenital syphilis over 90 per cent show a positive reaction if the disease be active.

5. In neurosyphilis the cerebrospinal fluid shows positive in all cases of general paresis but in only about 40 per cent of tabetics. Meningeal syphilis shows a high percentage of positive reactions. When syphilis is latent the cerebrospinal fluid sometimes shows a positive reaction while the blood Wassermann is negative. The many positive reactions reported in tuberculosis, scarlet fever, diabetes and other diseases are caused by an accompanying syphilitic infection.

In *advanced* sepsis, non-pulmonary tuberculosis, carcinoma, internal fungus infections jaundice or diabetes the blood serum is much changed and it may give a false positive reaction. If two or three positive reactions are obtained and the patient is not in a terminal state it may be necessary to apply a therapeutic test to decide on the presence of syphilitic infection. In tropical countries the test is robbed of some of its diagnostic value because positive reactions frequently occur in yaws, leprosy, trypanosomiasis, relapsing fever and malaria.

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[1] Cadham, F. T.: Canadian Med. Assn. Jour., August, 1922.

The positive Wassermann reaction is a more weighty indication for syphilis after the primary sore is two months old than any single physical sign. In chronic visceral syphilis all the signs are out of reach of our physical senses and are usually microscopic as well. It is here the Wassermann, which is positive in 90 per cent of cases, has its greatest value.

A positive Wassermann reaction is of more diagnostic value than a negative one. When a positive reaction is encountered the test should be repeated. If the reaction is again positive and none of the above listed diseases which cause non-specific reactions are present, the presence of syphilis may be taken as a certainty.

The Value of the Wassermann Test in Treatment.—In over 90 per cent sufficient treatment turns the reaction from positive to negative. A month should elapse between the completion of treatment and taking the test. If the treatment has been insufficient the Wassermann will remain positive or after being negative for a period of months will again turn positive.

The percentage that remain positive after routine courses of treatment are known as "Wassermann fast." They are uncured cases and persistence in intervals of medication and rest with a relay of arsenicals, bismuth and mercury will in time bring about complete clinical cure. The patient's general health and nutrition are equally as important as the medication. There should be no metallic poisoning. Treatment should be given with reserve to patients over fifty years of age.

Complete clinical cure is permanent freedom from symptoms and physical signs due to active syphilis. If the disease has reached the secondary stage before treatment is instituted latent histological evidence of syphilis may remain in patients who appear clinically cured. Occasionally the latent disease becomes active after many years because about 5 per cent of individuals have a poor resistance to syphilis and the usual medication does not produce the same striking result that it ordinarily does.

A Wassermann reaction which remains negative over a period of several years is our greatest criterion of cure. Other factors have to be considered. In early infections intermittent treatment extending over a year and in well established infection treatment for two years is usually necessary. Less treatment will only turn a Wassermann negative for about a year. Always when treatment is completed the Wassermann test should be performed twice yearly for at least two years and at yearly intervals for five or more years. A positive reaction is the earliest indication of a relapse of the disease.

The Report.—1. *A Negative Reaction.*—A negative reaction means (1) that the patient never had syphilis, (2) that the infection is too recent to have produced antibodies which give the reaction. (3) Temporarily negative after treatment. (4) That the syphilis is latent and inactive. (5) When the protective mechanism fails to appear to fight the inroads of the spirochetes.

2. *The Doubtful Reaction.*—The doubtful report shows 10 to 15 per cent reaction. It may be due to inferior technique, very early infection, diminished activity after treatment. The test should be repeated several times at weekly intervals.

3. *The One Plus Positive Report.*—This is a 25 per cent reaction. It occurs early in the disease and when the test is taken within several months after treatment. Several tests at weekly intervals should be done to rule out temporary causes and if the reaction is still one plus after several months further treatment should be instituted.

The 2 + Wassermann is 50 per cent positive, the 3 +, 75 per cent positive and the 4 +, 100 per cent. When two or three such reactions occur at weekly intervals a patient in temperate climate may be regarded as having active syphilis. Repeating the test is in the nature of an audit not only of the technique but also to rule out any temporary non-syphilitic cause that may produce a positive reaction in the patient.

CHAPTER IV.

EXAMINATION OF EXUDATES.

OUTLINE OF EXAMINATION OF PUS SMEARS.

Make 2 thin smears in every case. Dry them at once. Fix by moderate heat before staining.

Slide 1.

Stain with methylene blue for one minute. Wash, blot and dry, This stain gives the best outline of cells and bacteria. Examine for.

1. Type of cell: Polymorphonuclear.
    Monocyte.
    Lymphocyte.
    Epithelial cell.

2. Microörganisms: Morphology.
    Frequency.
    Intracellular or extracellular.

To classify bacteria when present use Gram's stain on Slide 2, as follows:

1. Crystal violet, one minute and wash.
2. Gram's iodine, one minute and wash.
3. Acetone to decolorize.
4. Safranin twenty seconds.

Gram-positive is purple. Gram-negative is red.

Gram-positive Bacilli.
Diphtheria. Bacillus welchii.
Tubercle. Malignant edema.
Boas-Oppler. Tetanus.
Subtilis. Anthrax group.
All other bacilli are Gram-negative.

Gram-negative Cocci.
Gonococcus.
Meningococcus.
Micrococcus catarrhalis.
All other cocci are Gram-positive.

EXAMINATION OF SMEARS OF EXUDATE.

Methylene Blue Stain. (*Loeffler's Alkaline.*)

Saturated alcoholic solution of methylene blue 15 cc.
1 in 10,000 solution potassium hydroxide in water 50 cc.

This solution keeps indefinitely.

To make saturated solution of methylene blue add 2 gm. of the powder to 25 cc. of concentrated alcohol (preferably methyl).

To make 1 in 10,000 potassium hydroxide solution add 0.1 of 1 cc. (2 drops) of a 10 per cent solution potassium hydroxide to 100 cc. distilled water.

Gram's Stain.

Crystal violet 0.5 gm.
Concentrated alcohol (preferably methyl) 50 cc.

This solution keeps indefinitely.

Gram's Iodine.

Iodine crystals 0.2 gm.
Potassium iodide 0.4 gm.
Water 60 cc.

This solution deteriorates slowly and turns light yellow in color. If it is not a deep red it should be renewed.

Decolorizing Solution.

Acetone or 95 per cent alcohol.

Safranin Counterstain.

Safranin powder 1 gm.
Water to make 100 cc.

This solution keeps indefinitely.

Pus Smears.—The value of a simple microscopic examination of stained pus smears can hardly be overemphasized.

Pus smears form a permanent record of the character and proportion of the organisms and cells as they actually exist in the lesion. They act as a control reference for bacterial cultures and vaccines made from them and one can detect if a virulent streptococcus is being crowded out by a less important colon bacillus.

Examination of the cells along with the bacteria tell the reaction of the body to the infection whether it be acute infection with its polymorphonuclears, subacute in which mononuclears predominate, chronic in which lymphocytes predominate or epithelial cells which indicate that the body tissues are not disturbed at all.

The most accurate quantitative measurements are done by the complement-fixation test for antibody content of the blood serum to the type of infection under study. The technique of this is quite involved and should be done in a special laboratory. Further advantages of smear examination are mentioned in Prognostic Value of Pus Smears, page 222, or Vaccines from Sputum, page 231.

General Remarks on Staining.—A clean wooden applicator, a steel probe or glass rod is satisfactory for making a smear. Use a small drop of pus and spread it out thinly in a patch about ½ inch square at one end of a glass slide. Dry the film immediately in the air and if convenient fix by heating the slide until it is as hot as the back of the hand can bear. Never send a drop of moist pus between two slides to a laboratory for diagnosis. The pus cells and bacteria disappear in an hour; turning into a homogeneous, gummy material which is of no value for diagnosis.

After the smear is fixed it makes for nice technique to circle the spread out exudate with a glass marking pencil and if more than one is being examined, mark the slide with the same number as that on a label with the patient's name. For washing off the stain use a gentle stream of water. The slide may be dried by using an ordinary blotting paper or waving it in the air. All stained specimens to be examined for bacteria require the oil-immersion lens. One should note first the type of cell present in several fields, then the bacteria, their shape, their frequency, their relation to each other, whether diplococci or diplostreptococci and also whether they are intra- or extra-cellular.

Methylene blue is by far the best stain for detecting bacteria. A search for Gram-negative bacteria is often very unsatisfactory because they are not easily seen. In the case of diplococci it is often essential to know whether they are Gram-positive or negative and here the Gram's stain should be applied on the second smear after the organisms are seen in the first smear stained with methylene blue.

In laboratories it is a good plan to have a bacterial mixture of Gram-negative colon bacilli and Gram-positive staphylococci and place a drop of this solution alongside of the smear to be examined as a control to tell if the stain is working properly. An improvised control may be made by removing a small amount of débris from between the teeth and placing it beside the smear to be examined. This material contains many Gram-positive and Gram-negative organisms.

The Gram's iodine solution is potent as long as it remains a reddish-brown and does not turn to a pale yellow. It should turn the violet stain purplish-black. In decolorizing, the acetone or alcohol is used drop by drop until the purple color ceases to flow away. Then portions of the smear should be colorless. The Gram-negative bacteria have lost the dye and to see them one must re-stain using a red color to distinguish them from the Gram-positive organisms which have retained the purple color. In a well-stained specimen the nuclei of the pus cells should retain little or none of the purple color.

Gram-positive.

(Deep purple).
Staphylococcus.
Streptococcus.
Pneumococcus.
Bacillus diphtheria.
Diphtheroid bacillus.
Bacillus xerosis (eye).
Bacillus of gas gangrene.
Bacillus of tetanus.
Bacillus of anthrax.
Tubercle bacillus.

Gram-negative.

(Red).
Gonococcus.
Meningococcus.
Micrococcus catarrhalis.
Colon typhoid group.
Bacillus pyocyaneus (green pus).
Bacillus of influenza.
Eye (Koch-Weeks bacillus, Morax-Axenfeld bacillus).

REGIONAL BACTERIOLOGY.

Infections of the Conjunctiva and Cornea.—Pus either as seen naked eye or under the microscope determines the presence of infection and examination of the stained smear under the oil-immersion lens will usually determine the causative organism. A bacteriological diagnosis is important in prognosis and treatment. The prognosis in gonorrheal conjunctivitis is not favorable because the cornea, especially in the adult, may slough away. Purulent conjunctivitis due to other bacteria has a better prognosis. As treatment in pneumococcal conjunctivitis or corneal ulcer 2 per cent optochin hydro-

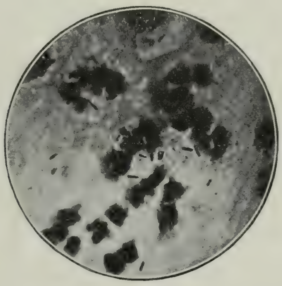

Fig. 47.—Secretion of muco-pus from conjunctiva in "pink-eye." × 1000 diameters. (Park and Williams after Weeks.)

chloride is very successful while it is of little or no value in other infections. In conjunctivitis due to the Morax-Axenfeld bacillus 0.5 per cent zinc sulphate is regarded as a specific.

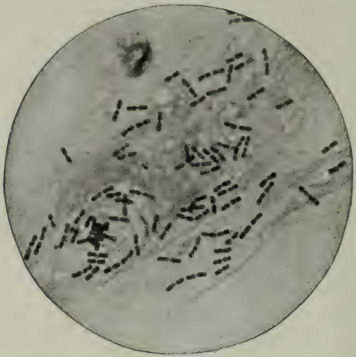

FIG. 48.—Morax-Axenfeld diplobacilli. (Todd and Sanford after Gifford.)

Method of Obtaining Smear from Conjunctiva.—Evert the lower lid and draw a sterile platinum loop or smooth steel instrument over the everted mucosa. Spread this material in a thin layer over $\frac{1}{4}$ inch area near one end of a glass slide. Masses of pus must be broken up and spread evenly. Make two slides, one to be stained with methylene blue and examined for cells and the presence of organisms. If organisms are present, a second slide is at hand to stain with Gram's method for differentiation of the bacterial groups. If these smears are to be sent to a laboratory, place them back to back, not face to face.

Interpretation.—Purulent conjunctivitis especially if it occurs during the first three weeks of life is frequently due to the gonococcus, and this organism is easily seen in the early infection.

Acute catarrhal conjunctivitis, sometimes called pink eye, is a contagious infection usually due to the Koch-Weeks bacillus, a small Gram-negative organism resembling the influenza bacillus in size and morphology. They occur in great numbers both within and among the pus corpuscles.

In the young, acute conjunctivitis is sometimes due to the pneumococcus. The pneumococcus is frequently the cause of corneal ulcers in the adult especially in those who also have dacrocystitis. Chronic catarrhal conjunctivitis is usually due to the large diplobacillus of Morax-Axenfeld which has rounded ends and occurs sometimes in short chains. The inflammation is most severe at the inner canthus of the eye. Bacillus xerosis, which is Gram-positive in contrast to the Morax-Axenfeld bacillus, is never pathogenic. Though staphylococci may be present in the conjunctiva normally, it is at times the cause of conjunctivitis. The presence of pus will decide this. The organisms are frequently so scanty that they can only be demonstrated on culture. Streptococci at times cause a purulent infection of the conjunctiva and the eye itself. Bacillus diphtheria which is an infrequent infection produces membranous conjunctivitis. Trachoma or granular conjunctivitis in its early stages occurs as many small granulations on the conjunctival surface of the upper lid. A search for trachoma bodies is of value. They appear as small granules near the nucleus of the epithelial cells in scrapings from the conjunctiva. Noguchi has isolated a small bacillus from trachomatous lesions but his cultural method requires special facilities to carry it out.

Organisms from the Throat.—Stain smear for Vincent's. (See page 233.)

Swab and culture for diphtheria. (See page 215.)

Swab from nasopharynx (through mouth) and culture for diphtheria, meningococci or autogenous vaccine. (See page 230.)

Swab from pharynx for tuberculosis when sputum cannot be obtained. (See page 224.)

Organisms from Ear.—Smear for pus and organisms.

Culture if necessary to determine type of pneumococcus or streptococcus.

Swabs from Wounds to Determine the Progress in Healing.—Many organisms indicate an active suppuration, few or no organisms indicate that recovery is well advanced and fewer dressings may be applied or the wound closed.

Urethral Discharges for the Diagnosis of Gonorrhea.—*In the Male.*—Diagnosis of early infection is established by a urethral discharge which shows microscopically pus cells and many Gram-negative diplococci.

The course of the disease is best followed by the two-bottle test.

Precaution.—The patient should not pass urine for two or three hours previous to the test so that any inflammatory products that may happen to be in the urethra will be seen.

The Test.—Three or 4 ounces of urine are passed in the first bottle, and the remainder in the second. Note if the specimens are clear or cloudy and if any shreds are present. On the first examination one should employ the microscope to make sure that any cloudiness is due to pus.

Types of urethritis.	First urine.	Second urine.
Anterior, early superficial.	Cloudy with shreds.	Clear.
Anterior to compressor urethra muscle, well established in crypts.	Turbid with shreds.	Clear with shreds.
Posterior with prostatitis.	Turbid.	Turbid.

Shreds are the masses of pus cells held together by mucus and discharged from the ducts of urethral mucous glands or prostate. This causes the pus cells to appear clumped under the microscope. These mucous glands harbor the infection longer than does the superficial mucosa of the urethra.

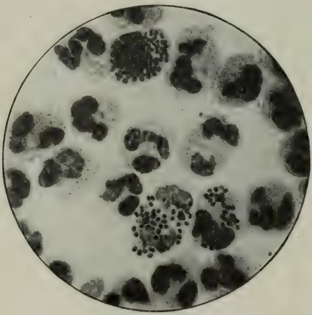

Fig. 49.—Gonococcus within pus cells, fuchsin stain. × 1000 diameters. (Fränkel and Pfeiffer.) (Park and Williams.)

This test is useful in determining whether a urinary infection in a female is localized to the urethra and cervix or whether the pus comes from the bladder and kidneys as a pyelonephritis.

To differentiate a bladder infection from a pyelonephritis cystoscopy is necessary. However certain differences are apparent from examination of the urine. In cystitis the fresh urine is alkaline and the pus content small but constant in every specimen passed during twenty-four hours. In pyelonephritis the urine is usually acid, the total pus content is large but it may be intermittent. By having the patient pass each voiding for twenty-four hours in separate bottles some will contain little while others contain much pus. When pus cells are present in the second specimen but no bacteria are in evidence even on culture of the specimen, tuberculosis should be suspected and tubercle bacilli sought.

In males some clinicians employ a three-bottle test in which the very last of the second sample is passed into a third container. The marked bladder contraction at the end of urination compresses the prostate, squeezing out some of its secretion. The emptying of the prostate by this means is not very reliable and it is better to use manual expression through the rectum and collect for examination the fluid that comes away from the urethra.

EXAMINATION OF PROSTATIC SECRETIONS.

Outline.—This examination may be done by expressing the prostatic contents and examining microscopically, the fluid which may be then obtained by stripping the urethra. The above mentioned methods will succeed in all but a few borderline doubtful cases where examination of the seminal emission collected in a male sheath obtains secretion from the deep recesses of the prostate gland that cannot be reached by manual expression. A few drops of 10 per cent acetic acid should be added to it immediately to prevent autolysis of bacteria.

Detail.—*Obtaining the Prostatic Fluid by Expression.*—Have the patient bend over so as to place the elbows on the knees or assume the knee-elbow position. Carefully introduce a well-lubricated gloved index finger into the rectum. At about 1 inch in the finger comes on the membranous urethra, which feels like half a lead pencil, at the center of the anterior wall. The normal prostate has its apex just above this and is a heart-shaped structure bulging slightly into the rectum. Normally it is slightly sensitive on moderate pressure. The lower part of the seminal vesicles when inflamed may be felt as rigid cords above and laterally to the prostate.

Note any areas of undue tenderness, bulging or firmness. Hold a clean container under the urethral orifice and express the contents of the seminal vesicles by pressing the finger above the upper and lateral border of the prostate. Next press slowly on the upper and outer border of the prostate. Move the finger its own width inward and press again. Repeat on the other side and finally strip the midline downward to express the secretion into the anterior urethra.

Microscopic Examination.—Add a drop each of 10 per cent acetic acid and methylene blue to the secretion and mix. The acetic will make the nuclei of any pus cells stand out. The dilute methylene blue will stain them faintly. Transfer with a platinum loop or pipet a small drop of this secretion to a glass slide, add a cover-glass and examine under the high dry lens, looking especially for pus cells with their clear cytoplasm and irregular nuclei. Do not confuse pus cells with the amyloid bodies or large degenerating granular cells which are normally present. Spermatozoa, lecithin bodies and epithelial cells are rarely seen when infection is present. Examine under oil-immersion lens.

Interpretation.—Up to 6 pus cells per microscopic field may be found in normal prostatic secretions and one cannot attach much significance to less than 10 cells. This is especially true if other elements such as epithelial cells, spermatozoa and lecithin bodies are present. If many pus cells are present one must look for the microörganism either by culture or direct bacterial examination.

Method for Bacterial Examination.—Add the prostatic secretions to a centrifuge tube of water and centrifuge. Pour off the supernatant fluid. Make a smear of the sediment. Stain and examine under the oil-immersion lens for bacteria. This procedure concentrates the pus cells and rids them of mucus and they do not shrivel up on the slide during the heat fixation.

Interpretation.—In subacute or chronic infection having acute exacerbations one may find the gonococcus. In chronic infections there are usually no organisms in the stained smear because they are too few. J. H. Hill saw organisms in 40 per cent of cases with chronic prostatic infection whereas he was able to obtain positive cultures in 70 per cent. The organisms were frequently Staphylococcus albus and colon bacillus, streptococcus occasionally and the gonococcus only rarely. An infected urine does not necessarily infect a prostate but an infected prostate will always contaminate the urine. Every posterior urethritis is a prostatitis. This examination is useful in detecting the presence of persisting gonorrheal infection.

When the gonococcus was the primary infection it is usually replaced by other pathogenic organisms in a year or two. There may be acute exacerbations or reinfection by the gonococcus however. Twenty per cent of the cases of chronic prostatitis are nongonorrheal in origin. They are probably secondary to tonsils, tooth and sinus infections. In looking for a focus of infection in the male the prostate should never be overlooked.

Chronic Gonorrhea in the Male.—Urethral discharge showing many pus cells, an occasional one of which contains several diplococci staining deep blue-black with methylene blue and are negative with the Gram stain would certify gonorrheal infection.

Last Stages.—After the disease has been present for several weeks, fewer of the pus cells will contain diplococci and only from one to three pairs will be present in each infected cell. Many diplococci lie outside of the cells. Mononuclear and epithelial cells appear as the disease becomes chronic. The morning is the best time to obtain smears and no treatment should be administered during the twelve hours previous to taking the smear as antiseptics cause a temporary absence of bacteria in the discharge.

In chronic urethritis a three bottle test will show

1st urine.	2d urine.	3d urine after prostatic massage.
Few shreds.	Clear.	Pus, occasionally gonococci.

Standard of Cure.—No microscopic pus on stripping the urethra. Clear urine in first and second specimens. Perhaps a few shreds in the first specimen. Prostatic secretion containing lecithin bodies, a few epithelial cells, sometimes spermatozoa and *less than* 10 *pus cells per high-power field* with no cocci.

Doubtful Cure.—Occasional pus cell with many epithelial cells on stripping the urethra. Many shreds in anterior urethra containing pus microscopically but no bacteria.

Prostatic secretion containing 10 to 100 pus cells per high-power field but no bacteria.

In less than 1 case in 20 do gonococci exist in the urogenital tract for over two years, and if pus is present longer than this it is usually caused by a secondary infection with diphtheroids or staphylococci, provided the patient has not been re-infected.

Other Valuable Diagnostic Procedures.—Urethral sounds, preferably with acorn tip;

Wassermann reaction for syphilis.

MICROSCOPIC EXAMINATION OF THE SEMEN FOR STERILITY.

While this may be done on semen collected in a male sheath it is probably best to proceed as outlined by Huhner which is as follows:

The wife presents herself for examination within an hour or two after coitus. If live spermatozoa are found in the posterior fornix or in the cervix the husband is not responsible for the sterility. Ropy muco-pus in the cervix acts as a barrier and kills spermatozoa. If the spermatozoa pass into the uterine cavity and are found alive at the fundus the cause of sterility is in the tubes or in the ovaries.

A long narrow glass pipet with a slightly curved tip and rubber bulb from a medicine dropper as an extractor is the most convenient instrument for obtaining the specimen. Examine the material between a slide and cover-glass unstained under the high-power lens with partially closed diaphragm. The spermatozoa in focus should not only be well formed with tails but they should be numerous and active. It is very necessary to prevent rapid drying of the specimen and the cover-glass is a great aid in this.

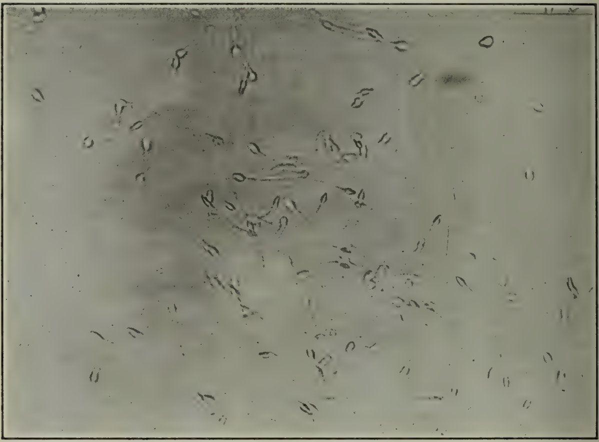

Fig. 50.—Unstained spermatozoa × 500 (under cover-glass and high dry lens). If healthy they are very active for twenty-four hours if kept warm and moist.

DIAGNOSIS OF GONORRHEA IN THE ADULT FEMALE.

1. **Urethra.**—Sponge off the urethral orifice and strip the urethra by inserting the gloved finger in the vagina, applying pressure upward against the pubes and bringing the finger down along the urethra. This will cause a drop of pus to exude from the urethra. Make smears. If pus cells are present but gonococci absent repeat the test on two successive days. Gram-negative intra-cellular diplococci are positive evidence of gonorrheal infection. This is the most reliable manner of diagnosing gonorrheal infection in the female.

2. **Cervix.**—Acute inflammation in the cervix of a woman who has never been pregnant is highly suspicious of gonorrheal infection. No douche or other treatment should be given for twelve hours prior to the test.

It is necessary to obtain secretion from *within* the cervical canal as vaginal secretion contains many confusing non-pathogenic bacteria. Insert a speculum and after sponging the cervix dry, transfer a small amount of pus from within the cervix to glass slides smearing it out in a thin layer. This examination should be repeated several times. In chronic gonorrhea, infection persists in the cervical canal after the urethritis has cleared up.

Other Signs of Gonorrheal Infection.

1. Venereal warts.
2. Inflammation of the paraurethral (Skene's) ducts.
3. Inflammation of the glands which lie between the lateral wall of the vagina and the labium minus (Bartholin's).
4. Salpingitis or ovarian abscess.

Standard of Cure.—No gross or microscopic pus on stripping the urethra and glands of Bartholin. Little or no cervical discharge. Smears taken preferably just before and after menstruation when no treatment is administered for the previous twenty-four hours should show epithelial cells, no more than a *few* pus cells and no Gram-negative diplococci. Gonococci are usually absent if the patient has a temperature over 100° F. They appear again when the temperature drops. This examination should be repeated several times before a physician can assure the patient that she has recovered from the infection.

Gonorrhea in Female Children.—Any acute vulvo-vaginitis is usually gonorrheal. Gonococci are present in the early stages but are hard to find in chronic cases so that one may regard discharges of pus from the vagina of a child as gonorrheal in origin even when no bacteria are seen. It is a very persistent infection and very contagious to other children.

DIAGNOSIS OF SYPHILIS FROM SUPERFICIAL LESIONS.

Caution.—No antiseptic dusting powders should be applied for twenty-four hours previous to the examination and arsphenamin or mercury at any time previously will cause the Treponema pallidum to disappear from the lesions.

Obtaining the Specimen.—Wear rubber gloves for this procedure. Large numbers of Treponema pallida are present in the early active lesions of syphilis, such as chancres, mucus patches, condylomata and swollen lymph glands. The Treponema is *in* the tissue, other spirochetes are *on* the surface. Cleanse the lesion with sponges moistened with water, then dry off the surface. Squeeze the sore until a drop of serum appears. Transfer this fluid to the slide. There should be no visible hemorrhage as numerous blood cells interfere with the examination.

Obtaining Material from Enlarged Lymph Glands.—Insert a 20-gauge needle into the center of a lymph gland. Inject 5 drops of sterile 0.9 per cent saline solution and massage the gland. Attach the syringe to the needle and apply suction for a minute. Withdraw the needle and smear the aspirated material on several slides for examination. If the material is to be sent to a laboratory for dark-field examination it is best to collect in a capillary tube and seal the ends with the flame.

Material from Lesions in the Mouth.—Clean the surface thoroughly to avoid the dental spirochetes which may resemble the Treponema pallidum. Apply suction with the tip of a tuberculin syringe.

Examination of Exudates for Treponema Pallidum.—*Choice of Methods.*—The dark-field method is the best because large areas can be examined and the corkscrew motion of the spirochete which is of diagnostic importance is seen by the same principle as particles of dust are seen in a sunbeam. It has several disadvantages however. The cost of the apparatus is considerable and for satisfactory work a microscope should be set up solely for use of dark-field work as adjusting the dark field takes considerable time. Examination by this method requires more skill than by stained methods.

The stained method supplies a permanent record of the finding but one can discover only 90 per cent as many positive findings as by the dark field. On the whole the dark-field method is more suited to the large laboratory. The stained method is more suitable for the practitioner with a small laboratory.

Staining Methods.—The Treponema pallidum is extremely resistant to ordinary stains and therefore special methods have to be employed. Wright's stain modified by adding slightly alkalinized water instead of distilled and allowing this to act for fifteen to thirty minutes will show spirochetes a faint pink and other structures a deep blue. The best method is the modified Fontana's stain outlined on page 233.

One should stain and examine a control smear from between the teeth to make sure that the technique is satisfactory immediately before staining for Treponema pallidum.

Distinctive Characteristics of the Treponema Pallidum Under the Oil-immersion Lens.—The Treponema pallidum is a very delicate structure having 5 to 10 sharp spirals which appear as tiny zigzags in the stained specimen. About 5 of these spirals are equal to the diameter of a red blood cell in contrast to other spirochetes and spirilla which are coarser, have a more elongated spiral and fewer turns.

Interpretation.—A positive finding is of the utmost importance. It means a very early diagnosis which makes treatment more effective. Although the syphilitic hard sore does not appear until more than a week after exposure, sometimes chancroidal ulcers which appear a few days after exposure contain Treponema pallida, a double infection. Only by an examination of the exudate can one determine this.

BACTERIAL CULTURES OF EXUDATES.

The Thermos Bottle as a Substitute for an Incubator.—*Apparatus.*—Culture tubes containing solid media. Loeffler's blood serum media is best. Convenient sterile tubes containing media and swabs devised by Bass may be obtained from supply houses. They are hermetically sealed and so do not deteriorate.

Thermos bottle.
Ordinary bath thermometer.

The Test.—Every sample of exudate sent for culture should be accompanied by a smear of it on a slide. The only possible exception to this is in the diagnosis of diphtheria where the culture is more efficient than the smear. Even in taking cultures for diphtheria one should be on the lookout for Vincent's angina because its grayish membrane resembles that seen in diphtheria. In Vincent's infection a fever occurs only in a few very acute cases. The diagnosis is made wholly from the smear as the spirillum and the fusiform bacillus will not grow on ordinary culture media.

Inoculate the media tubes by the swab containing the material to be cultured. Fill the thermos bottle three-fourths full of water warmed so that the thermometer immersed in it will register 103° F. This will gradually drop to 96° F. in ten hours but forms a satisfactory incubator for the overnight growth of diphtheria bacilli and in fact nearly all pyogenic organisms. Place a rubber band around the top of the inoculated culture tube, tie a thread to the band and lower the tube so that it will float with its lower end immersed in the warm water. Cork the bottle leaving the end of the string out and set in a warm place overnight. Next morning remove a platinum loopful of the culture, spread it on a glass slide, stain and examine under the oil-immersion lens.

DIPHTHERIA BACILLI, LOEFFLER'S METHYLENE BLUE STAIN.

Diphtheria bacilli are slender, sometimes slightly curved, with dark-staining ends and sometimes a dark dot in the center of the longer forms. The dark dots at the ends of the bacilli serve to distinguish diphtheria from the non-pathogenic diphtheroids which are shorter and plumper and take on a uniformly intense stain. Barred forms of bacilli are rarely found as the sole type of bacillus in the throat of those suffering from diphtheria. They are very frequently found in normal throats.

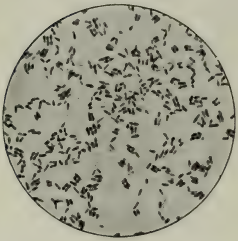

FIG. 51.—Pseudo-diphtheria bacilli. (B. hofmanni.) (Park and Williams.)

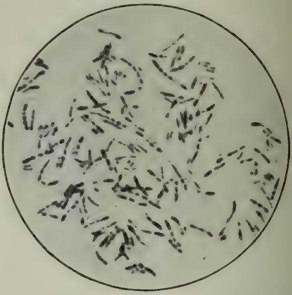

FIG. 52.—Bacillus diphtheriæ, methylene blue stain. × 1000. (Kendall.)

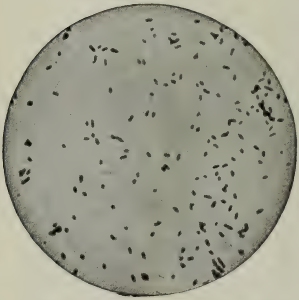

FIG. 53. — Bacillus pyocyameus. (Park and Williams after Kolle and Wassermann.)

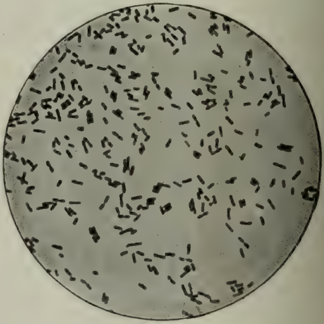

FIG. 54.—Colon bacilli. Twenty-four-hour agar culture. × 1100 diameters. (Park and Williams.)

Culture for Differentiation of Bacilli in Colon Typhoid Group (Gram-negative).—Urine or a thin emulsion of feces to be tested is spread on Endo's special plate media. Colon grow as pink colonies. Typhoid-dysentery colonies are grayish.

Isolated colonies obtained by culture on ordinary media are pure cultures and may be subjected to the four culture method (Kolmer). To distinguish the special type of dysentery bacillus further, cultural methods given in texts on bacteriology should be used.

Organisms.	B. coli group.	B. cloacæ group.	B. lactis aërogenes group.	Dysentery group.	Friedländer's group.	Typhoid group.	Paratyphoid group.	Proteus group.
Litmus media	Pink	Pink	Pink	No change	No change	No change	No change	No change
Broth for motility .	Motile	Motile	Non motile	Non-motile	Non-motile	Motile	Motile	Motile
Glucose media	A. & G.	A. & G.	A. & G.	Sl. A.	A. & G.	A.	A. & G.	A. & G.
Gelatin media	No liq.	Liq.	No liq.	No liq.	No liq.	No liq.	No liq.	Liq.

A = acid; G = gas; Liq. = liquefaction.[1]

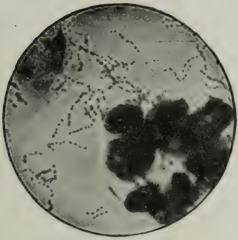

Fig. 55.—Streptococci in peritoneal fluid, partly enclosed in leucocytes. × 1000 diameters. (Park and Williams.)

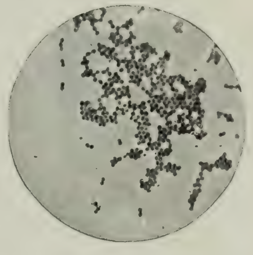

Fig. 56.—Staphylococcus. × 1100 diameters. (Park and Williams.)

DIFFERENTIATION OF STREPTOCOCCI.

Thin smears of the exudate will reveal the presence of streptococci. Short chained organisms, although occasionally virulent, are the usual type found in normal throats. The long chain types (10 to 20 cocci) are more likely to be virulent. The large capsule of Type 3 pneumococcus may be recognized when present.

There are three main groups of streptococci which may be differentiated by culture on blood agar.

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[1] Kolmer and Boerner: Laboratory Diagnostic Methods, D. Appleton & Co.

1. Streptococcus hemolyticus which has a clear zone of hemolysis around the colony.
2. Streptococcus non-hemolyticus has no hemolysis.
3. Streptococcus viridans produces a small greenish colony.
4. The pneumococcus produces a small green colony but the organisms are bile soluble and will ferment inulin.

The streptococcus hemolyticus is the commonest cause of acute septic disease and is only found in 10 per cent of healthy throats. It is the cause of non-diphtheritic sore throat, scarlet fever, meningitis, mastoiditis empyema, peritonitis, cellulitis and erysipelas. Specific serum agglutination reactions show that scarlet fever and erysipelas each have a special strain of hemolytic streptococcus. It is only where the organism has been typed serologically that antistreptococcal serum is of value. Polyvalent antistreptococcal serum has failed to prove itself of any value. *Non-hemolytic streptococcus* is usually found in healthy throats and bowel contents. It may produce mild disease.

Streptococcus viridans occasionally found in healthy throats is usually the cause of serious subacute or chronic focal infections of tooth, tonsil or sinus. It is likewise found in gastric and duodenal ulcers and in subacute bacterial endocarditis, when it may be recovered by a blood culture.

CHAPTER V.

PUNCTURE FLUIDS.

PLEURAL, ASCITIC AND OTHER FLUIDS.

Obtaining the Specimen.—A good trocar, size 12 to 15, with perforated cannula should be used rather than a large needle with bevelled point which irritates tissues in the cavity and readily clogs up. Other essentials are 1 per cent novocaine solution, hypodermic needles and a small sharp lance to incise the skin. Mercurochrome or iodine for the skin, sterile instruments, gloves and towels are essential.

Thoracentesis should be performed when the amount of fluid is large enough to reach the clavicle or displace the heart and mediastinum; also when moderate amounts have formed so rapidly as to disturb the patient by unduly rapid breathing or rapid heart action or cyanosis. It should be repeated when necessary. The site of puncture is in the eighth intercostal space which is just below the lower tip of the scapula. After local anesthesia and a small skin incision the trocar is inserted close to the lower border of the seventh rib. Less than an inch of needle usually enters the pleural space and the instrument should not be pushed further to irritate the lung. Withdraw the fluid slowly. A liter or more may be removed.

Aspiration should be stopped if the patient has a severe paroxysm of coughing, pain or oppression in the chest, a feeling of faintness, or a blood-streaked sputum.

Removing Ascitic Fluid.—The best site for paracentesis of the abdominal wall is midway between the umbilicus and the pubes. The patient should have the bladder empty and be in the sitting position. Aseptic precautions, local anesthetic, a skin lance and a trocar with fenestrated cannula are necessary. Provided the bowel is not adhered to the abdominal wall from a previous operative wound in the center line, there is no danger of puncturing the bowel wall. In fact it is difficult to drive a trocar into a loop of bowel held closely in the hand. The patient should have an abdominal binder which should be tightened as the fluid leaves the abdominal cavity. Do not remove the fluid quickly.

Diagnostic Puncture of the Pericardium.—The best site of puncture is in the fifth left intercostal space just outside the nipple line. This is usually ½ to 1 inch inside the lateral border of relative dullness and no apex beat should be palpable at this point. Push the trocar in slowly, tilting it upward and medially to meet the apex. If it touches the heart before fluid drains away, heart movement is felt on the needle and it should be withdrawn. This is not a dangerous procedure and it should be practised more frequently than at present. In cases of marked effusion 200 to 500 cc. may be removed.

PUNCTURE FLUIDS.

CLEAR SEROUS TRANSUDATE.

Diagnostic	**Light yellow or greenish.** **Specific gravity below 1.018.** **Sero-mucin negative (Moritz).**
Further study shows	**Albumen under 3 per cent.** **Smear of sediment—mostly endothelial cells.** **No bacteria.**

SEROFIBRINOUS EXUDATE.

Diagnostic	**Yellow, cloudy, with strands of fibrin.** **Specific gravity above 1.018.** **Sero-mucin positive (Moritz).**
Further study shows	**Albumen over 3 per cent.** **Smear of sediment—mostly lymphocytes.** **No bacteria found although process is usually due to tubercle bacilli.**

PURULENT.

Diagnostic	**Direct smear shows many pus cells and pneumococci, streptococci or Bacillus coli.**
Further study shows	**Special culture and serum reactions show type of organism, i. e., Type I pneumococci or hemolytic streptococci.**

Examination of Clear Fluid.—*Specific gravity* with urinometer as outlined for urine.

Sero-mucin (Moritz).—To a test-tube full of the fluid add 2 drops of 5 per cent acetic acid. *A heavy white precipitate* indicates an inflammatory origin. *A slight opalescence* indicates a transudate.

Quantitative Albumen.—Dilute 1 part of the fluid with 10 of water and use Esbach's test (page 310) or the sulphosalicylic method (page 280).

Cytodiagnosis.—To prevent clotting add a pinch of citrate or oxalate to a test-tube full of fresh fluid and centrifuge for five to ten minutes.

Examination is not very satisfactory when a clot has formed. Spread out a small portion of the clot on a slide, fix and stain it with methylene blue or a blood stain. Cells are contained in the meshes of the fibrin. Make a differential count of the lymphocytes, endothelial cells and polymorphonuclears recording the percentage of each in 100 or 200 cells and note bacteria if any are seen.

Interpretation.—The transudate resulting from chronic heart or kidney disease or obstruction of vessels is a watery fluid with a specific gravity below 1.018, negative sero-mucin test and albumen content of less than 3 per cent and the cells in the sediment show a preponderance of large endothelial cells.

Serous or serofibrinous exudate, usually due to tuberculosis, is a semi-transparent fluid with a specific gravity over 1.018. It shows a positive sero-mucin test, and has over 3 per cent of albumen. Microscopic examination of the sediment shows the predominating cell to be a lymphocyte.

The opaque purulent exudate is usually due to a pneumococcus, streptococcus or colon bacillus, contains polymorphonuclears and the bacteria responsible for the inflammation. A stained smear of a drop of the pus will usually determine this. At times it is valuable for therapeutic purposes to know the type of pneumococcus or streptococcus. Culture and serum reactions are then required. Hemorrhagic and chylous fluids are rare. Although the hemorrhagic fluids were thought to be all due to malignancy, careful investigation has shown that a tuberculous process is occasionally responsible for hemorrhagic fluids. It is quite common to find in a clear fluid due to a tuberculous process, many lymphocytes, a few polymorphonuclears and red cells. The tubercle bacilli are rarely found.

A malignant process cannot be diagnosed with any degree of accuracy from examination of the sediment of the fluid. Large vacuolated cells, said to be diagnostic of carcinoma are found in chronic inflammatory processes of long-standing. *The most suggestive feature of malignancy* is an unusual frequency of cells showing mitotic figures. Hemotoxylin shows the nuclei most clearly. Chylous fluids are most often the result of advanced nephritis. Occasionally it is due to actual erosion of the chylous ducts.

Valuable Prognostic Observation in Peritonitis. (Wilkie.)—Make a smear of pus from any case of acute peritonitis, stain with methylene blue for a minute and observe under the oil-immersion lens, (*a*) the character and proportion of the cells whether polymorphonuclear or monocytic phagocytes.

(*b*) The number of bacteria present and the degree of phagocytosis by the cells.

Many degenerated polymorphonuclears and many bacteria free from the cells show lack of phagocytic power and the patient will die. Many monocytic phagocytes, a few polymorphonuclears and a few bacteria within the cells show a high degree of phagocytosis and the outlook is good.

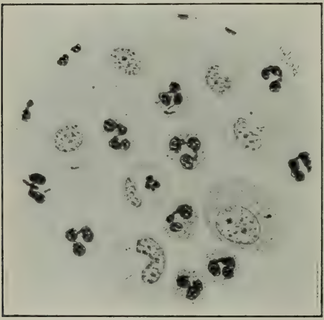

Fig. 57.—Film from a case of general peritonitis, successful phagocytosis; numerous mononucleated macrophages. (Wilkie.)

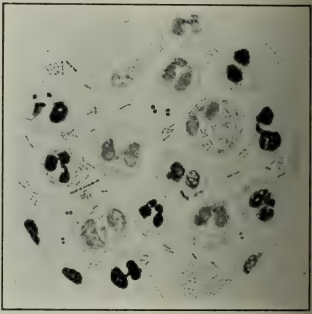

Fig. 58.—Diffuse peritonitis from appendicitis; active but inadequate phagocytosis; fatal.

This examination may be made in two minutes after an abdominal laparatomy and not only determines the prognosis but is a guide to the type of operation done. Where phagocytosis is good and few bacteria seen, no drainage and little postoperative support is necessary. Where the bacteria are many and are not phagocytized by the polymorphonuclears, drainage is advisable and all the postoperative support possible such as transfusion, appropriate specific serums, etc., should be applied.

CHAPTER VI.

EXAMINATION OF SPUTUM.

OUTLINE OF SPUTUM EXAMINATION.

ROUTINE.

Amount in twenty-four hours. Time at which specimen is produced.

GROSS EXAMINATION. **APPEARANCE.** **UNUSUAL ODOR.**

- Mucoid.
- Muco-purulent.
- Purulent.
- Blood-tinged.

MICROSCOPIC EXAMINATION STAINED BY THE ACID-FAST METHOD.

Stain: (1) Hot carbol fuchsin—three minutes.
(2) Acid-alcohol—to decolorize.
(3) Methylene blue—one-half minute.

Note:

CELLS.	BACTERIA.
Epithelial.	Tubercle bacilli.
Monocytes.	Pneumococci.
Pus cells.	Streptococci.
	Influenza bacilli.
	Mixed organisms.

Special:

- Typing of pneumococci.
- Preparation of autogenous vaccine.
- Examination for elastic fibers and blastomycoses.
- Examination for Vincent's organisms.

EXAMINATION OF SPUTUM IN DETAIL.

Collection of Sputum.—Supply the patient with a *clean* wide-mouthed bottle and a new cork. Instruct him to collect for examination only the morning sputum coughed from the lungs. Secretions from the nose should not be included.

It is well to examine the specimen as soon as possible, noting the appearance, whether

Mucoid.
Muco-purulent.
Purulent.
Blood-streaked.

Also note any unusual odor and obtain a rough idea of the amount coughed up in twenty-four hours by having the patient use sputum cups.

Examination for Tubercle Bacilli.—*Apparatus and Reagents.*—New glass slides, Petri dishes or paper sputum cups, toothpicks or wooden applicators or forceps, marking pencil for glass.

Carbol fuchsin (Ziehl-Neelsen[1]).
Acid alcohol.[2]
Methylene blue—Loeffler's alkaline (see page 203).

Pour the sputum into a Petri dish or flattened-out paper sputum cup. Fish out with forceps or two clean wood toothpicks small opaque, purulent or cheesy particles of the sputum and put them on one end of a glass slide. Squeeze them out with another glass slide until the layer of sputum is thin and even, covering about one-half the slide. Fix the smears over a flame being careful not to brown the sputum. Now mark the slide with a number corresponding to the one on the name slip. Destroy the specimen.

Staining.—Principle.—The tubercle bacilli have a fatty covering which is not easily penetrated by stains so heat is necessary. Once stained, it holds its color when washed with alcohol and mineral acids while the other structures lose theirs.

Technique.—Pour enough carbol fuchsin on the slide to cover the half of the slide on which the sputum is spread. Holding the dry

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[1]

Absolute alcohol	10 cc.
Basic fuchsin	1 gm.
5 per cent aqueous solution of phenol	90 cc.

Dissolve the fuchsin in the alcohol. Add the phenol solution and filter the following day.

[2]

Acid alcohol-hydrochloric acid concentrated chemically pure	3 cc.
Alcohol, 95 per cent	97 cc.

end of the slide by hand, heat the end covered by stain until steam appears. Never boil the stain. Add fresh stain over any dry spots. The same degree of staining may be obtained by leaving smears in cold stain overnight. After three minutes rinse the stain off with water. Decolorize the smear with acid alcohol until it is a pinkish-gray on washing with water. Counterstain with Loeffler's methylene blue for one-half minute. Wash, then dry with blotting paper and heat. Examine under the oil-immersion lens.

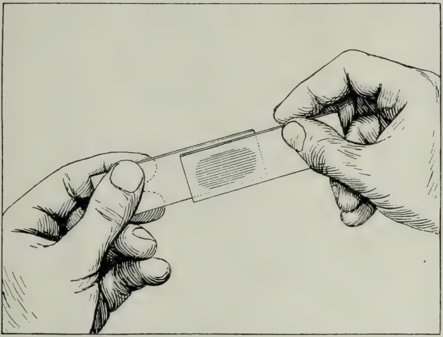

FIG. 59.—Spreading out a thick particle of sputum for microscopic examination.

Sputum Examination.

Patient's Name............................Doctor................

Date..

Character Mucoid................
Character Muco-purulent.........   Amount.............
Character Purulent...............

Cells found Epithelial cells..............
Cells found Pus cells....................

Bacteria found Tubercle bacilli..............(five minute search under the microscope)..

Examined by....................

Examination.—After examining a field carefully move the slide systematically to view other fields so that a large area of the smear will be examined. Five minutes should be spent on this part of the examination. Definite small red rods are tubercle bacilli. Some are slightly curved, others slightly beaded. Only after seeing several should a positive diagnosis be made. Reddish bacilli in thick reddish areas should be regarded with suspicion if not seen also in thin parts of the smear. Occasionally a small mark in the glass will retain the red stain. Record the type of cell present and the approximate number of bacilli per microscopic field.

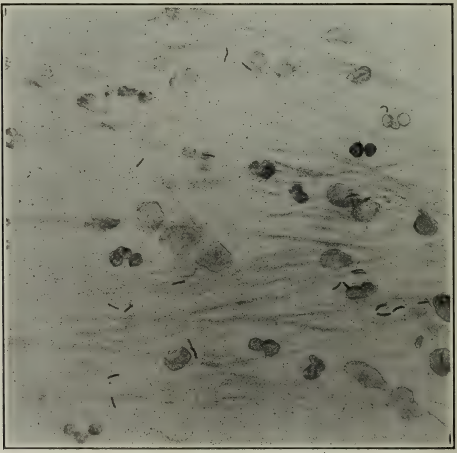

Fig. 60.—Tubercle bacilli × 1000. With the Ziehl-Neelsen stain, the rod-like bacilli are red and the cells blue.

Interpretation.—The finding of even three or four definite acid-fast bacilli in the sputum means pulmonary tuberculosis which has broken down and discharged its contents into a bronchus. A positive finding should be confirmed by examination of other specimens. Repeated examinations are indicated in suspicious cases as the tubercle bacilli may not be present at all times. The occurrence of one or two tubercle bacilli organisms only, on one examination when all other evidence is against tuberculosis is of doubtful significance. The sputum should be examined four or five times and if bacilli are present they will be seen again.

Waiting until tubercle bacilli are found in the sputum before making the diagnosis of pulmonary tuberculosis is to jeopardize the patient's chance of recovery. At times however bacilli in the sputum are the very first sign. When sputum is not available, swabs from the pharynx have at times shown tubercle bacilli. (Lawrason Brown.) Failure to find tubercle bacilli does not rule out pulmonary tuberculosis. When many pus cells but no bacilli are seen it suggests that the disease is due to the pneumococcus or streptococcus.

After the diagnosis of pulmonary tuberculosis is made it is very important to distinguish between the closed case where there are no bacilli discharged in the sputum and the open case where they are being discharged and the patient is liable to infect others. The prognosis of the patient who has tubercle bacilli in the sputum is worse than the tuberculous patient whose sputum does not show them. It is estimated that only 25 per cent of patients with pulmonary tuberculosis live for ten years after the bacilli are found in their sputum.

Sources of Error.—Smears too thick may retain the acid-fast stain and show reddish bacilli which are not tubercle. Scratches in the slide and cracks in a charred sputum may retain hardened stain. Some food particles, wood fibers or any bacilli may be acid-fast if they are coated with vaseline when a poorly cleaned vaseline bottle is used as a container.

Probably no other test is more frequently performed in an unsatisfactory manner than the examination of sputum for tubercle bacillus. Picking out a suitable mass of sputum and spreading it on a glass slide is a nasty job if the examiner does not fully realize the value of finding tubercle bacilli when they are present. It happens all too frequently in sputum examinations that a platinum loop or wooden applicator is run through the specimen of sputum. Thick masses are raised but slip back into the container leaving a small drop of mucus on the wire loop. Not the mass of pus but this mucus is spread in a rough fashion on the slide. And now other mistakes are liable to happen such as browning the sputum in fixing, boiling the stain instead of merely steaming it, allowing the stain to dry off, or decolorizing too long. Then the examination under the microscope may be less than one-half minute and a report rendered. Only where the specimen is swarming with bacilli will one succeed in finding them using poor technique. The clinical diagnosis is usually obvious in such a case. The early doubtful case with sputum showing only a few bacilli requires the best available technique if the organisms are to be found.

It is necessary to have more than 100,000 bacilli to each cubic centimeter before any are detected in the stained smear under the microscope with good technique (Corper). Is there any wonder that bacilli are frequently missed when one or more of the errors above enumerated occur in the examination?

Examination of Sputum for Pyogenic Bacteria.—To examine the sputum for ordinary pathogenic bacteria such as the pneumococcus streptococcus or influenza bacillus, staining thin smears with methylene blue or Gram's stain and examination under the oil-immersion lens is routine. As the influenza and pertussis bacilli stain poorly with ordinary stains, it is best to dilute 1 part of carbol fuchsin with 10 of water and apply this diluted stain for five to ten minutes. In culturing special media containing fresh blood is necessary for their growth.

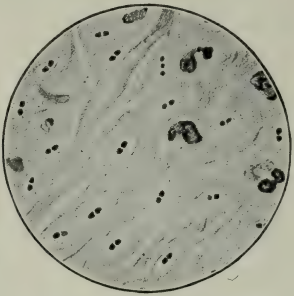

FIG. 61.—Pneumococcus in sputum. × 1000. (Kendall.)

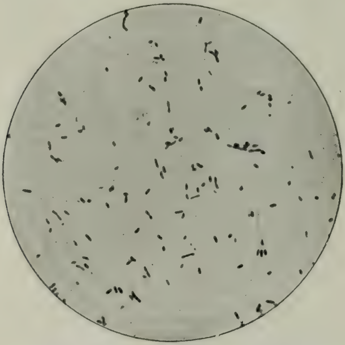

FIG. 62.—Influenza bacilli. × 1100 diameters. (Park and Williams.)

Interpretation.—It is generally agreed that the pertussis bacillus is the cause of whooping cough. The pneumococcus is the cause of lobar pneumonia. While the streptococci and influenza undoubtedly cause respiratory infections the evidence shows that in common colds and influenza they are secondary invaders after an ultramicroscopic filter-passing organism has attacked the tissues.

Type Determination of Pneumococcus. Rapid Method.—*Material and Apparatus.*—Five-cubic-centimeter vials of Type I, II and III serum. These may be obtained from any biological house.

Six small test-tubes.

Three 1-cc. pipets.

Two centrifuge tubes.

Centrifuge.

Specific precipitins appear in the urine twelve hours after the initial chill in 60 per cent of cases.

Place half a cubic centimeter of Type I, II and III respectively in 3 small test-tubes. Overlay with clear urine if the patient has had the infection for at least twelve hours. A faint cloud to a heavy precipitate at the line of juncture of the urine with the serum indicates the type of pneumococcus responsible. The reaction may take place in a few minutes at room temperature or it may require five to fifteen minutes incubation in a water-bath 50° to 55° C. This test is so easily performed that it should be tried when conditions warrant it.

Coagulation Method for Type Determination (Krumweide).—This may be applied to thick sputum, spinal fluid, blood, pleural, peritoneal and other exudates.

Examine the sputum or exudate microscopically to identify the pneumococci which appear as Gram-positive, slightly elongated small cocci frequently occurring in pairs or short chains.

A hyaline capsule is usually present around pneumococci in exudates. There is an unusually heavy one around Type III form which is also known as pneumococcus mucosus. As Type III has the highest mortality (about 45 per cent) it has been suggested that the capsule protects the bacteria against the antibodies of the host. If pneumococci are present in large numbers microscopically transfer 1 to 3 drams of sputum or exudate to a centrifuge tube. Immerse in boiling water for several minutes until a firm clot results which will only occur if the sputum is from the lungs and contains albumin. Break up the clot with a glass rod or pipet. Cork the tube and shake vigorously to dissolve out extracts from the bacteria. Centrifuge at a high speed for ten minutes. Pipet off the clear supernatant fluid which is used for the test. If not enough is available add 0.5 to 1 cc. of water to the sputum. Shake again and centrifuge. The addition of water is rarely necessary.

Place 0.2 to 0.3 cc. of each of the type serums in a narrow test-tube marked with the number corresponding to the type of serum it contains. Overlay the serum in each tube with an equal amount of the clear extract (antigen) from the sputum. This is best done by tilting the tube and allowing the fluid to run from a pipet slowly down the side of the tube.

Place the tubes in a water-bath 50° to 55° C. for five minutes. If the specimen is satisfactory and contains Types I, II or III pneumococci, a ring of precipitate will occur at the line of contact of the extract and serum of the corresponding group. If definite precipitation is obtained with any one of the type sera one need not proceed further.

Where there is only a small amount of antigen in the sputum the ring will develop more slowly and will be less marked. Place in water-bath for an additional fifteen minutes. A true ring is opaque and is best seen against a black background.

If still in doubt shake the tubes and incubate for one-half hour. A definite clouding throughout is positive. When numerous typical pneumococci are present on microscopic examination but no precipitin reaction occurs, there is either a failure of the reaction or the organism is Group IV. More certain methods require injection of the sputum washings into a mouse and eight hours later the pure culture obtained from the peritoneal cavity is tested for agglutination with the type sera.

To confirm Group IV in which no serum reactions occur pneumococcal cultures are soluble in one-fifth the quantity of 10 per cent ox bile. Streptococci are not. Group IV is made up of a number of definite types.

Serum Treatment of Pneumonia.—A serum has been prepared for Type I pneumococcus infections. It reduces the mortality by one-half. One hundred cubic centimeters of Type I serum given intravenously should be administered at an early stage in the disease and the dose repeated every eight hours until the temperature stays under 100° F. Determine the patient's reaction to the serum before administering it. (See page 352.)

At present most of the polyvalent antipneumococcus serums are fairly potent in Type I antibody but weak in the others. The use of such a serum cannot be advised. Polyvalent serums will only be advisable when we can procure highly concentrated solutions like Felton's with the antibody potency of each type affixed in units. The initial dose of 10,000 units may be given when the clinical diagnosis is made and injections repeated only when the pneumococcus is Type I or II.

COMPARISON OF DEATH-RATES IN TREATED AND UNTREATED CASES.[1,2]

	Treated.			Untreated.		
Type.	Cases.	Died.	Mortality, per cent.	Cases.	Died.	Mortality, per cent.
I	152	31	20.4	144	45	31.2
II	102	40	39.2	102	53	52.0
III	38	14	36.8	55	15	27.2
IV	137	35	25.5	121	39	32.2
	429	120	28.0	422	152	36.0

The death-rate from Group III pneumococcus is around 45 per cent in some series investigated.

Preparation of Autogenous Vaccine from the Sputum.—It is difficult to obtain a pure culture and vaccine of the organisms causing disease in the walls of the bronchi. Care must be taken to avoid non-pathogenic organisms from the nose, mouth or container which may

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[1] Patients who died within twenty-four hours after admission are omitted.

[2] Cecil and Sutliffe: Jour. Am. Med. Assn., 1928, **91**, 235.

contaminate the specimen. The morning sputum is best. If it is scanty 5 grains of potassium iodide given the previous evening will make it more copious.

Certain other precautions are necessary. A clean sterile container, oral toilet with the tooth-brush and mouth-wash followed by a rinse of tap-water. Then the sputum from the lungs is coughed up and deposited into the container. Postnasal droppings should not be included.

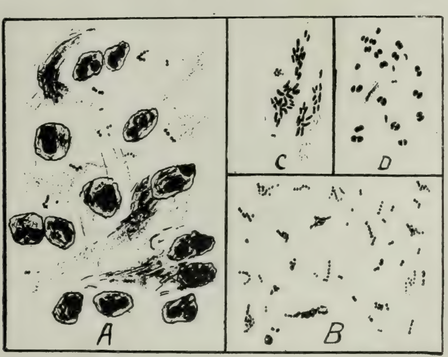

Fig. 63.—Suitable organisms for vaccine. *A*, a typical sputum from a patient with asthmatic bronchitis; *B*, a drop from a carefully prepared vaccine containing the same type of organism as the sputum; *C* and *D*, unsuitable vaccines, from organisms in the throat which contaminate sputum and multiply rapidly.

The saprophytic organisms grow many times as rapidly as do the disease-producing ones, and, if fresh sputum be not used and sub-culture be not resorted to, the saprophytic will crowd out the pathogenic bacteria. An important principle is that the organisms from a drop of the vaccine should be of the same character as those in the sputum coughed up from the lungs. The streptococci and pneumococci are most important from an etiological standpoint. While on culture these groups vary considerably among themselves, any practitioner can determine gross defects in a vaccine by comparing under the microscope a drop of it stained on a glass slide with a stained smear of the patient's fresh sputum. This examination requires only a microscope, methylene blue stain, glass slides, and less than ten minutes of time. If the sputum is to be sent to a laboratory at some distance, a smear on a slide should accompany it, as the bacteriologist can better determine the more important organism from the smear. The diphtheroids and Micrococcus catarrhalis, which grow even at room temperature, would make an unsatisfactory vaccine for a patient whose sputum contained the organisms shown in *A* of Fig. 63. A vaccine containing the organisms outlined in *B* of Fig. 63 would be satisfactory. When a patient has bronchiectasis many organisms develop in the dilated bronchi and complicate the problem of vaccine preparation, but those of the streptococcus group should be sought. The dosage should cause a moderate local reaction, but should not be large enough to aggravate the patient's symptoms. He should receive 10 tô 15 injections at intervals of five to seven days. Such efforts to heal chronic bronchial infection give, at present, the best hope for relief.

Examination of the Sputum for Elastic Fibers and Fungus Infections.—In cases which appear to be clinically tuberculosis but whose sputum shows no tubercle bacilli, examinations should be made for elastic fibers, streptothrix, ray fungus, blastomycosis, and other fungi.

These structures may be seen in the unstained specimen and are best examined by placing the thicker masses of sputum on a slide by means of tooth-picks. Add a drop of 10 per cent potassium hydroxide and press down under a coverslip. Examine under the low and high dry lens.

Elastic fibers are highly refractile and smooth but may be frayed or curled at the ends. They may be in coiled bundles or in a honeycomb design as in the alveolar formation of the lung. This arrangement serves to distinguish them from cotton fibers which are flat irregular strands, usually twisted on themselves. Elastic fibers occur in acute destruction of the lung as in cavity formation.

Fungi.—Streptothrices occur as numerous fine, branching filaments which swell at their ends. They may be stained by the method outlined for ringworm in hairs.

Ray Fungus.—Pour the pus into a test-tube of water. Examine microscopically the yellowish-white granules which fall to the bottom. By pressing them between two slides and examining under the high-power lens the circular-ray arrangement consisting of rods are seen to radiate from a caseous center. This also may be stained by the method outlined for ringworm in hair.

Blastomycosis.—Blastomycosis is a rare disease produced by round or oval budding cells which are pathogenic yeasts that affect the lung much like tuberculosis.

Spirochetes in the Sputum.—Spirochetes are more common in sputum than was previously thought. They are frequently found in the sputum of supposedly tuberculous patients whose sputum shows no tubercle bacilli. They are quite frequent in patients who have pulmonary hemorrhages but rarely in those with bronchial dilatation or pneumothorax. The normal habitat of these organisms is on the teeth just at the gum line, in the tonsil crypts and about the genitals. The spirochete infections occur as Vincent's angina, noma, pyorrhea alveolaris, putrid otitis media, putrid pneumonia and lung gangrene and the gangrenous processes that rarely occur about the genitals known as the fourth venereal disease. Predisposing factors are usually of first importance in determining the development of these organisms.

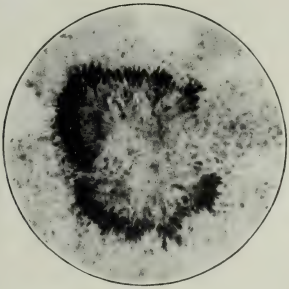

FIG. 64.—Actinomyces colony showing peripherally arranged clubs. (Kendall.)

In searching for this organism it is necessary to instruct the patient to clean the teeth and mouth, which may harbor spirochetes, before raising the sputum. It should be examined within half an hour after expectoration.

Cerquir's Modification of Fontana's Stain.—*Reagents.*

1. Alcohol 90 per cent.
2. Mordant.—Tannic acid chemically pure, 5 gm.
   Glacial acetic acid, 4 cc.
   Alcohol (90 per cent), 50 cc.
   Distilled water, 50 cc.
3. Carbol fuschsin, Ziehl-Neelsen.

1. Fix the smear by flooding with 90 per cent alcohol for one minute. Drain.

2. Without drying add the tannic acid mordant, heating it over a flame until a little vapor is given off.

3. Wash in water.

4. Cover with carbol fuchsin and heat to steaming for ten to twenty seconds.

5. Wash, dry and examine under the oil-immersion lens. The spirochetes appear a pinkish violet.

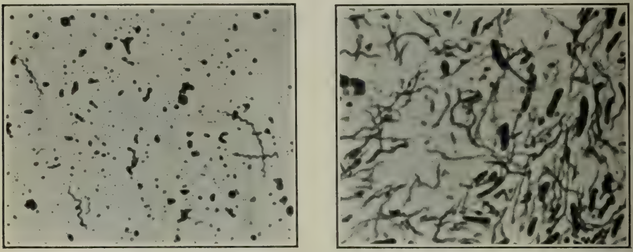

Fig. 65.—Left, spirocheta pallida from a primary syphilitic sore. Right, spirilla of Vincent which occasionally contains an organism with sharp curves. Cerquir's modification of Fontana's stain. Oil-immersion lens × 1000.

Albumin in the Sputum.—To 1 cc. of sputum add 5 cc. of 10 per cent acetic acid. Filter and add a pinch of sodium chloride. Heat to coagulate the albumin or estimate its percentage by Esbach's method.

Interpretation.—Normally only a trace of albumin is present. In chronic bronchitis and asthma slightly more is present. The sputum of tuberculous or pneumonic patients contains 0.1 to 0.3 per cent of albumin.

CHAPTER VII.

EXAMINATION OF THE CEREBROSPINAL FLUID.

Origin.—The cerebrospinal fluid originates mainly from the choroid plexus in the lateral and fourth ventricles of the brain. The pressure of fluid is about equal to the venous pressure but only about one-sixth of the intracranial arterial pressure.

Experimentally small beads of fluid have been seen appearing on the surface of the choroid plexus which acts as a semi-permeable membrane so that the fluid is a dialysate from the blood plasma. The flow may be reversed back into the blood stream by an increase of the osmotic pressure of the blood as in dehydration or ingestion of concentrated salt solutions. In inflammation of the meninges the capillary cells are disorganized and allow protein substances, which they normally hold back, to pass into the cerebrospinal fluid.

Circulation.—An adult has 100 to 150 cc. of cerebrospinal fluid which circulates from within the cavities of the brain to its surfaces. The ventricles are all connected by foramina and the fluid escapes from the fourth ventricle into the cisterna magna, which is below and behind the brain. Here the course of the fluid divides, four-fifths of it going up over the brain and one-fifth down around the spinal cord. The fluid which goes to surround the brain flows anteriorly beneath the medulla, pons and mid-brain. From here it passes up along the internal carotids and spreads in the sulci which cover the surface of the brain. The smaller portion of fluid descends around the spinal cord and there is a small upward flow in the central canal of the cord itself. An increased amount of fluid in the subarachnoid space causes increased cerebrospinal fluid pressure which produces a compensating rise in the venous pressure. This in turn causes a rise in the general blood-pressure to maintain the circulation of the cerebrospinal fluid. Blocking of the fluid's exit from the ventricles produces internal hydrocephalus.

Absorption.—The main absorption is by osmosis into the blood capillaries in the cerebral sulci. Absorption also occurs into the Pacchionian bodies which are arachnoidal villi formed by reduplication of the arachnoid into the great venous sinuses especially along the superior longitudinal sinus. In the spinal canal some fluid is absorbed into the vessels in the subarachnoid space and the lymph spaces. The diffusion into the blood keeps pace with the formation of the fluid because the total content of salts and other electrolytes in the blood is greater than in the cerebrospinal fluid.

In meningitis or injury where there is much albumin in the spinal fluid this absorption is less rapid and results in an increased amount and pressure of spinal fluid. Repeated lumbar puncture drainage is therefore a logical procedure to bring the pressure and amount of such a fluid more nearly to normal.

OUTLINE OF CEREBROSPINAL FLUID EXAMINATION.

LUMBAR PUNCTURE.

NEEDLE DEPTH.—Adults 2½ to 3 inches, children 1 to 2 inches.
NORMAL PRESSURE RECLINING.—100 to 200 mm. of water (7 to 15 mm. Hg.)
Children.— 50 to 100 mm. of water.
Do Queckenstedt's test.

EXAMINATION OF FLUID.

FOAM TEST—Shake in small tube.
Normal—slight foam disappearing in one minute.
Pathological—much foam persisting after five minutes.

COAGULUM FORMATION.—Do not shake. Set fluid aside in a test tube for twenty-four hours. Coagulum develops only in pathological fluids.

CLEAR FLUIDS.

CELL COUNT.—Place a drop of undiluted spinal fluid in the counting chamber. Count under the low-power lens, the cells in the whole Neubauer ruled field. Result multiplied by $\frac{10}{9}$ equals cells per cu.mm. Normal 0 to 5.

DIFFERENTIAL COUNT.—(If more than 10 are present).—Centrifuge 2 or more cc. Pour off the fluid, pipet the sediment to a glass slide, dry and fix. Stain with methylene blue or Wright's and under the oil-immersion lens find the percentage of lymphocytes, red cells and polymorphonuclears in 100 cells.

GLOBULIN.—Overlay with 1 cc. of spinal fluid ½ inch of saturated ammonium sulphate solution in a small clean tube. White line at the junction indicates an excess of globulin.

WASSERMANN.—Send 2 cc. to the central laboratory.

CLOUDY FLUIDS.

Place 1 drop of the fluid on a glass slide. Fix, stain with methylene blue and examine under the oil-immersion lens for cells and bacteria. Examine another slide stained by Gram's method. If no bacteria are present culture the fluid.

ROUTINE EXAMINATIONS:

NOTE:

- **(a) Pressure.**
- **(b) Color.**
- **(c) Foam on shaking.**

Examine 10 cc.

Cloudy Fluids for (a) Types of cells.
(b) Bacteria.

Clear Fluids for (a) Cell count.
(b) Globulin.
(c) Wassermann reaction.
(d) Coagulum formation.

EXAMINATIONS OF OCCASIONAL VALUE:

- **(a) Collodal reactions.**
- **(b) Sugar percentage.**
- **(c) Chloride percentage.**
- **(d) Urea percentage.**
- **(e) Protein content.**
- **(f) Differential precipitation test (Levinson).**
- **(g) Nonne-Apelt reaction.**

EXAMINATIONS RARELY INDICATED:

- **(a) Cisternal puncture.**
- **(b) Ventricular puncture.**
- **(c) Ventriculography and encephalography.**
- **(d) Lipiodol injection in spinal canal.**
- **(e) Brain puncture.**

Apparatus.—Lumbar puncture needle. A needle of small bore such as 22-gauge is best for routine work. It should be 3 to 3½ inches long and of *non-breakable material.* Steel needles are too easily broken and should never be used. Nickel is suitable but gold or platinum although expensive is best. The needle should have a short bevel and be sharp. It should have a good stylet and the hub should fit the nozzle of a record syringe and be marked on the side which has the bevelled point. A 19-gauge needle should be on hand where there is a "dry tap" due to purulent meningitis.

Manometer.—The simple water or spinal fluid type is best. The manometer devised by Ayer is marketed by Codman and Shurtleff, Boston, and consists of 2 tubes each 30 cm. long and 2 mm. in diameter with a stopcock. An improvised outfit may be made of 60 cm. (2 feet) of rubber tubing with a very small lumen (2 mm.) like a duodenal tube. One end is attached to a stopcock which will fit the hub of the needle. The other is attached to a small glass tube which is held up to the level that the spinal fluid rises in the tube. The distance measured with a tape from the needle to the fluid level is the pressure of the cerebrospinal fluid in millimeters of water.

Local anesthetic. Novocaine in tablets or ampules. Mercurochrome or iodine solution, rubber gloves and sterile wipes and towel.

Contraindications for Lumbar Puncture.—The main contraindication is tumor at the base of the brain, especially below the tentorium. Release of the fluid below by lumbar puncture results in a sudden pushing down of the brain on the medulla. This causes a cessation of respiration and other vital functions. The most reliable sign of tumor at the base of the brain is papilledema or choked disk as seen by the ophthalmoscope. It is usually safe to proceed with lumbar puncture in cases of brain tumor if there be no papilledema or choked disk. Care should be taken to release the fluid slowly and remove only 5 cc.

General contra-indications for lumbar puncture are advanced disease of the heart or arteries.

Obtaining the Fluid.—*Position of the Patient.*—The patient should be in bed, lying on his side with his knees drawn up toward his chin. This bows out the spine and separates the vertebræ leaving more space for the needle to enter. The site of needle puncture may be determined by placing a slight daub of iodine at the crest of the iliac bones and a line joining these passes between the third and fourth lumbar spines which is the best place for lumbar puncture.

Sterilized skin, sterile rubber gloves, sterile towels and sterile needles should be a rigid rule for every lumbar puncture.

Anesthetic.—It is very important to use local anesthetic. Under its effects the procedure is robbed of its terror and we are able to conduct this valuable examination without objection on the part of the patient. Use 1 per cent novocaine and 1 in 100,000 adrenalin for every puncture except on comatose patients or the insensitive tabetics or paretics.

Place the index finger of the left hand between the third and fourth lumbar spines. The point of entry for the needle is ½ inch below and ½ inch to one side of the index finger mark. Instil the first few drops *between* the layers of the skin. This is the most sensitive area through which the needle passes. Then infiltrate the muscle for about 2 inches with a dram of anesthetic solution in the direction in which the lumbar puncture needle will pass.

Inserting the Needle.—After five minutes again press firmly with the index finger of the left hand between the third and fourth lumbar vertebræ. Hold the needle with its point ½ inch below and ½ inch to one side of the first finger. Have the bevelled side of the needle toward the patient's side so the point will split the many longitudinal fibers. Then insert it *slowly,* slightly upward and inward so that when the point is 2 to 3 inches deep it will be directly under the left index finger. By this the heavy interspinous ligament is avoided. The dura should be pierced at 2½ to 3 inches. The signal of this is the marked *lessening of resistance* to the needle as the ligamentum flavum is passed. Once the dura is punctured the fluid should come away on removing the stylet. After a few drops come away attach the manometer and estimate the pressure as described on page 240. The patient should have his head straight and breathe freely.[1]

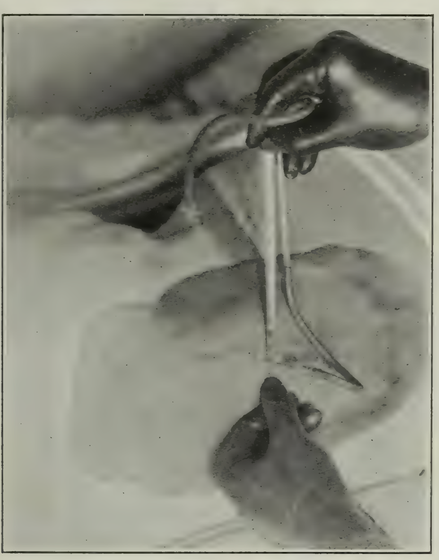

Fig. 66.—Lumbar puncture. The needle is inserted and the pressure is being estimated by measuring the height of the fluid as it rises in the glass tube.

Collection of Fluid.—Collect the fluid in 2 sterile test-tubes as the first tube may contain blood caused by the needle puncture. For routine examination of a clear fluid 10 cc. is ample and no more should be removed. Mark the first tube. The fluid in it may be used for all other examinations. It is necessary to do the cell count within six hours as the cells disappear rapidly after that time. Where there is meningitis and increased intracranial pressure removal of 25 to 40 cc. gives relief to the patient. It should be slowly removed and stopped when the pressure becomes two-thirds of its initial reading or if the patient's pulse or respiration shows any irregularity. The pressure normally drops about 50 mm. after 10 cc. of fluid is withdrawn. When there is an excess of fluid as in hydrocephalus, serous or true meningitis, the drop is less than one-half that of a normal person. On the other hand in tumor a drop of 50 mm. may occur after 5 cc. is removed. This may occur where no disease is present however. When the necessary amount of fluid is collected remove the needle and sponge off the site of puncture with alcohol. No dressing is necessary. The natural scab which forms makes for the quickest healing. Collodion plasters tend to produce a skin infection by confining to the puncture, sweat which is always infective, rather than allowing it to evaporate.

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[1] If no fluid appears turn the needle half a turn so as to cut through the dura. Should this maneuver fail, push the needle ⅛ inch further and rotate it another half turn. Do not push the needle in so far that it will pierce the nerve roots and cause a sharp pain in the leg or come to the body of the vertebræ which has a profuse venous plexus overlying it. In case of failure, withdraw the needle and try again. Should this fail, another puncture may be attempted between the second and third lumbar spines, or if acute meningitis is suspected, a large needle may be used to allow the thick pus to come away. It is best to first observe a skilled operator, then attempt the puncture the first few times under his supervision. Practice on a cadaver is valuable.

After Care. Headaches.—The patient should drink 5 to 6 glasses of water during the following few hours and remain in the horizontal position for twelve hours after a lumbar puncture.

Severe headache used to be a common sequel to lumbar puncture. It can be almost wholly avoided by (1) using a fine sharp needle which produces a small incision in the dura that readily closes. Formerly a coarse blunt needle was frequently used and it produced a large rent which allowed the spinal fluid to drain through the dura into the lumbar muscles for several days. (2) Having the patient remain quietly in the prone position for some hours following the puncture so that a minimum of internal pressure is exerted on the dural puncture favors the sealing up of the rent. (3) Drinking a liberal supply of water which will soon replace the spinal fluid drawn off. Should headache occur, pituitary extract hypodermically affords relief.

Pressure with Water Manometers.—With the patient in the horizontal position from 100 to 200 mm. is the normal pressure. Over 250 mm. is definitely abnormal. From 200 to 250 mm. is suspiciously high. When the patient is upright the spinal fluid pressure readings are twice those quoted for the prone position. No cause can be ascribed to abnormally low readings.

While mercury manometers are more compact they do not show small variations. Mercury is $13\frac{1}{2}$ times as heavy as water. Therefore the mercury pressure multiplied by $13\frac{1}{2}$ equals the water pressure.

The pressure is slightly greater on expiration than inspiration. The fluid level should fluctuate with respiration normally. Holding the breath or coughing produces a sudden and rapid increase due to a distention of the veins to the brain and spinal canal. Pressure on the neck over the jugular causes a distention of the veins in the brain and an instant rise in the spinal fluid pressure. This is known as Queckenstedt's phenomena and is due to the venous back pressure displacing the cerebrospinal fluid. If no rise takes place on pressure over the jugular there is a blocking of the spinal subarachnoid spaces due to *meningitis, tumors of the cerebellar* fossa or double and complete lateral sinus thrombosis. A fluid which rises but does not fall to the previous level or even a slow return is strong evidence of a partial block of the spinal sub-arachnoid space probably by a ball-valve-like tumor or exudate. Lateral sinus thrombosis may be demonstrated by pressing on each jugular vein alternately. When no increase takes place in the spinal fluid pressure it indicates a complete lateral sinus thrombosis on that side. The spinal fluid pressure drops about 50 mm. after 10 cc. is withdrawn. When there is an excess of fluid as in hydrocephalus, serous or true meningitis, the drop is less than one-half this amount.

In recent years a determination of the spinal fluid pressure has been found more valuable than was formerly believed. This estimation when made by observing the rate of flow was very misleading. At times when the fluid comes away drop by drop the pressure is quite high. The pressure which is very great in cerebellar tumor may cause the fluid at first to spout out and then after 5 or 10 cc. comes away it may quickly fall to normal because the tumor blocks further drainage from the cranial cavity. An estimation of the pressure should be made by a water manometer in every case.

APPEARANCE.

The fluid may be:

1. **Water clear.**
2. **Blood-tinged.**
3. **Clear yellow.**
4. **Cloudy.**

1. **Clear.**—The normal cerebrospinal fluid is as clear as water. Many abnormal spinal fluids however are also clear as in tuberculous or syphilitic meningitis and poliomyelitis. Occasionally such pathological fluids show a slight opalescence or have a few tiny flakes of fibrin. On standing a fibrin coagulum develops.

2. **Blood-tinged Fluid.**—Blood-tinged fluid may be the result of:

(*a*) Rupture of a subdural vein by the needle.

(*b*) Previous sub-arachnoid hemorrhage which may be due to (1) violent injury, (2) spontaneous sub-arachnoid hemorrhage resulting from rupture of an aneurism on the circle of Willis, or (3) an apoplectic hemorrhage into a ventricle.

It is extremely important to differentiate the accidental hemorrhage occurring during puncture from the truly pathological varieties. Blood caused by the needle puncturing the sub-arachnoid venous plexus of the spinal canal tends to disappear after the first few drops of fluid come away provided the injury produced was not a large one. Blood resulting from head injury or intracranial disease is diffuse and the fluid in the second 5 cc. container is as much blood tinged as in the first.

3. **Clear Yellow.**—It is possible to recognize hemorrhage occurring previous to the puncture by the color of the fluid after the cells have been settled by the centrifuge. Immediate trauma from the needle imparts no yellow color unless the amount of blood is very large (25,000 red cells per cu.mm.). If the hemorrhage has existed for over three hours a slight yellow tinge known as xanthochromia will develop in the supernatant fluid. A more intense yellowish-red color develops in the fluid if the hemorrhage has existed more than a week. This usually disappears in two or three weeks and the fluid again becomes colorless if hemorrhages have not occurred in the interval. Similar yellow fluids occur as a result of venous stasis produced by a tumor, abscess, high Pott's disease, cerebral tumor or thrombosis. There is also an accompanying high-protein content which may coagulate spontaneously. This is known as the Froin syndrome.

4. **Turbid Fluids.**—Turbidity is usually due to a great increase in pus cells but occasionally it may be almost entirely due to bacteria. There are usually over 400 pus cells per cu.mm. when turbidity is present whereas an equal number of lymphocytes causes no turbidity at all. Such a cloudy fluid is positive proof of acute meningitis due to meningococcus or other pyogenic organisms. Early in the disease the fluid is only slightly cloudy but later it may become more purulent and opaque.

Cloudy Fluid Examination for Acute Pyogenic Infection.—Place a drop of fluid on a glass slide, fix with heat and stain with methylene blue. Examine under the oil-immersion lens for:

(*a*) Type of cells.

(*b*) Bacteria.

Stain another smear with Gram's stain to differentiate the cocci. Organisms will be found in the following order of frequency:

Meningococci, intracellular very few present, Gram-negative.

Pneumococci, capsule usually present, Gram-positive.

Streptococci, no capsule present, Gram-positive.

Tubercle bacilli, in fibrinous web of clear fluid, acid-fast.

If cells are scanty it may be necessary to centrifuge 5 cc. and examine the sediment. If many pus cells are present but no bacteria are seen, incubate the fluid for six to twelve hours.

Interpretation.—The types of cells are very important. The polymorphonuclears, when present are usually in large numbers and signify an acute pyogenic infection. Lymphocytes and other mononuclear cells are never numerous enough to make a fluid cloudy. They signify a chronic infection such as tuberculosis, syphilis or subacute poliomyelitis. A few polymorphonuclears are present in the acute form.

Bacteria are usually seen with the polymorphonuclears in the acute infections and the character of the organism is quite definite. Occasionally the meningococci are very scanty in the early stages of the disease. Under such circumstances it is necessary to incubate the fluid in which the organisms grow profusely. When cocci are found the Gram stain should be applied as meningococci are always Gram-negative. To give the patient the benefit of prompt treatment it is justifiable to inject antimeningococcus serum when the spinal fluid is purulent but no bacteria are seen.

A culture of the spinal fluid will soon determine the ultimate value of such treatment. Only in Type I pneumococcal meningitis is serum of any value. Recently Kolmer[1] has advised for pneumococcal meningitis injection into the carotids and cisterna magna of pneumococcus antibody and 0.75 cc. of 1 per cent optochin HCl. He advised 20 cc. of 0.5 per cent acriflavin base into the carotids for streptococcal meningitis. Serum is of no value in streptococcal meningitis. It has a bad prognosis. Daily lumbar puncture will relieve the intracranial pressure. Should fibrinous adhesions prevent the downward flow of spinal fluid, a cisternal or ventricular puncture should be considered.

CLEAR FLUID EXAMINATION FOR SUBACUTE OR CHRONIC DISEASE.

1. **Foam test.**
2. **Clot formation.**
3. **Cell count.**
4. **Globulin content.**
5. **Wassermann reaction.**

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[1] Jour. Am. Med. Assn., 1929, **92**, 874.

1. **Foam Test.**—A marked foam on shaking which persists for more than five minutes is the result of an increased amount of protein in the fluid and therefore only occurs in pathological fluids. On shaking a normal spinal fluid the foam layer is thin and disappears in a few minutes.

2. **Clot Formation.**—This is a gelatinous pellicle that forms in pathological fluids on standing. If there is a high-protein content the pellicle forms in an hour. In tuberculous meningitis or in poliomyelitis, standing overnight is required and the pellicle is an extremely delicate fibrin web with cells in its meshes. Tubercle bacilli are not easily found in the spinal fluid of a patient who has tuberculous meningitis but if 5 cc. of the fluid be set aside overnight then the pellicle floated out on a slide, dried and stained it gives the best chance of finding the bacilli.

In the more chronic meningitis, tabes and poliomyelitis no pellicle forms although small white flakes may be present. Shaking the fluid, as may occur in transport, will prevent clot formation. A very large accidental admixture of blood due to the puncture may in itself produce a clot.

3. **Cell Count.**—The cell count is the most important single examination of a spinal fluid, and much attention should be concentrated on a good technique. The count should be made as soon as possible after the fluid is withdrawn because the cells disappear after standing six hours. A drop of formalin added to each cubic centimeter of spinal fluid will inhibit the cytolytic process. It interferes with the Wassermann and colloidal reactions however.

Method.—Shake the test-tube containing the spinal fluid last removed so as to distribute the cells evenly. Transfer, by means of a pipet or glass rod, a small drop of fluid to the counting chamber. After the cells have settled, count them under the low-power lens, covering all the ruled area which in the Neubauer ruling consists of 9 sq. mm., 3 on each side. As the chamber is $\frac{1}{10}$ mm. deep this gives a cubic content of $\frac{9}{10}$ of a cu.mm. To estimate the cells in a cu.mm. divide the number counted by 9 and multiply by 10. Two separate drops should be counted and their average taken. Normal 0 to 5 lymphocytes. If more cells are found they may be either *red cells, lymphocytes, mononuclears* or *polymorphonuclears* and it is important to know which type of cell is responsible for the increase.

It is important to use this method rather than the older methods which employ dilution with acetic acid. If a few blood cells are present acetic acid will hemolize them leaving an increased number of white cells which may be regarded as the result of meningeal disease instead of slight hemorrhage, probably traumatic.

Method of Differential Cell Count (Greenfield and Carmichael).—To 0.5 cc. of well mixed spinal fluid in a test-tube add 0.05 cc. (1 drop) of 0.2 per cent watery solution of methyl-violet. Mix thoroughly and allow to stand ten to fifteen minutes for staining of the cells. Shake again to distribute the cells evenly in the fluid and place a small drop by means of a pipet or glass rod on the counting chamber. Examine under low-power lens for total count and under high-power lens for differential count. If difficulty is experienced in distinguishing lymphocytes from red cells 0.05 cc. (1 drop) of 10 per cent acetic acid should be added to the stained spinal fluid in the test-tube and the test repeated.

In calculating, for the dilution when a drop of the stain alone is used in 0.5 cc. of spinal fluid and the cells in 9 sq. mm., $\frac{1}{10}$ mm. deep are counted and found to be 28, multiply by $\frac{10}{9}$ for cells in a cu.mm. and by $\frac{11}{10}$ for the dilution with stain or $\frac{12}{10}$ where both stain and acetic acid are used.

Example.—$28 \times \frac{10}{9} \times \frac{11}{10}$ equals number of cells per cu.mm.

If instead of adding a drop of stain to 0.5 cc. of spinal fluid the leucocyte pipet is used the stem filled to 1 with stain (equal parts of methylene blue or methyl-violet and weak acetic acid) and the spinal fluid drawn to "11" multiply by 10 and divide by 9 for dilution by the stain.

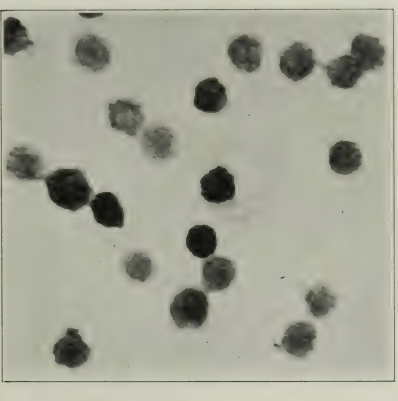

FIG. 67.—Lymphocytosis in the spinal fluid from a case of tuberculous meningitis. × 1000.

If the special diluting fluid is not at hand, centrifuge the spinal fluid; then stain and examine under the oil-immersion lens a drop of the resulting sediment for a differential count of the mononuclear and polymorphonuclear cells.

Interpretation.—The normal cell count in the cerebrospinal fluid is 0 to 5 per cu.mm. One cannot however attach much significance to a count of under 10 cells. A cell increase is very definite evidence of neurological diseases but as it occurs in many of them, it is not diagnostic of any one. As a principle, up to 50 lymphocytes may be taken as evidence of a chronic infection. A greater increase mainly lymphocytes is due to a bacillary or spirochetal infection. An increase of over 500 cells, 90 per cent polymorphonuclears is due to an acute coccal infection. There are many findings not covered by this principle and a number of exceptions to it.

Occasional increases of up to 20 lymphocytes per cu.mm. take place in herpes zoster, polyneuritis, chronic encephalitis, disseminated sclerosis and brain tumor. These are diseases in which there is usually no cell increase. When up to 200 cells composed of 90 per cent lymphocytes are present in an acute meningeal disease it is highly suggestive of tuberculous meningitis and it warrants prolonged search of the fibrin clot for tubercle bacilli. From 10 to 100 lymphocytes per cu.mm. with a positive Wassermann reaction in the spinal fluid or blood is pathognomonic of tabes, paresis or meningo-vascular syphilis. The higher counts occur with the acute stages while in the later stages of tabes and paresis the count may be little above normal and the disease is not improved by antiluetic treatment as is vascular syphilis. In poliomyelitis there is an increase of from 50 to 500 cells mainly polymorphonuclears at the beginning of the disease. After a week the polymorphonuclears are replaced by lymphocytes which persist for two or three weeks.

Over 500 cells consisting of more than 90 per cent polymorphonuclears indicates acute meningitis due to one of the cocci. The count may be up to 2000 when the fluid is definitely purulent.

Frequently a mixture of cells are encountered, the polymorphonuclears making up 10 to 25 per cent of the whole. It is difficult to draw deductions from such findings. The total number of cells may vary from 10 to 200 per cu.mm. depending on the extent and severity of the lesion. The lower counts may occur in lateral sinus thrombosis, brain abscess or softening near the surface or near the ventricle, occasionally in degenerating brain tumor, while the higher mixed counts may occur in syphilitic or tuberculous meningitis.

In oozing brain abscesses or extension from otitis media, or nasal sinusitis producing a localized meningitis there may be several hundred or more cells, more than 75 per cent polymorphonuclear. Bacteria are usually absent even on culture provided the meningitis does not become general.

An excess of cells is usually associated with an excess of globulin but the globulin content may be normal and the cells increased in disseminated sclerosis, poliomyelitis and encephalitis lethargica.

Fractional Examination of the Cells.—Cell counts from the first and last fluid usually vary. In spinal disease the cell count in the first 5 cc. is highest while in disease involving the brain only, the highest cell counts occur after 50 cc. of fluid is removed (Kafka).

More exact localization of cell increase may be arrived at by comparison of the fluid obtained by lumbar, cisternal and ventricular puncture.

Cell counts of the cerebrospinal fluid at monthly intervals are useful in following the progress of neurological disease. They may be made at monthly intervals in chronic disease or every few days in acute disease. A lowering of the cell count usually indicates a favorable course although in very chronic paresis or tabes the mere draining lowers the cell count and the actual improvement is not in accord with the decrease in cells.

4. **The Globulin Test.**—*Reagent.*—Saturated solution of ammonium sulphate.

Preparation.—Dissolve 85 gm. of chemically pure neutral ammonium sulphate in hot water. As it cools some of the salt will crystallize out leaving the supernatant solution a saturated one. After cooling filter it and test with litmus. It should be neutral. This may be used for the test and if tightly stoppered it keeps indefinitely.

The Test.—Overlay 1 cc. of saturated ammonium sulphate solution in a small clean test-tube with spinal fluid from a 1-cc. pipet in the same manner as the nitric acid ring test is applied for albumin in the urine. A fine white line as thin as a sheet of paper at the juncture of the two fluids indicates more than a normal amount of globulin. The thicker the ring, the larger the amount of globulin present. If the tube is shaken a general turbidity is produced.

Interpretation.—The presence of increased amounts of globulin has very much the same significance in a spinal fluid that albumin has in the urine. Globulin is most constantly found in the spinal fluid of patients who have meningitis, the amount increasing with the severity of the disease. The largest amounts are due to meningitis resulting from meningo-, strepto-, pneumo- or staphylococci while the smaller amounts are present in tuberculous meningitis, cerebral syphilis, general paralysis, tabes dorsalis and early anterior poliomyelitis. There is usually a cellular increase proportional to the globulin increase.

Excess globulin is also found where there is intracranial hemorrhage or where a tumor produces a venous stasis resulting in an exudation of blood protein into the spinal fluid. In this compression syndrome there is little or no cellular increase although the protein and globulin content may be so great as to cause spontaneous coagulation of the fluid on standing. This is especially true in subtentorial and spinal tumors. It may occur in paraplegia due to myelitis, polyneuritis or in cerebral tumor.

The Wassermann Test.—Over 2 cc. of fluid should be submitted for the Wassermann test. This test requires ten times as much spinal fluid as does the test on blood serum.

Interpretation.—A positive reaction in the cerebrospinal fluid signifies syphilitic disease. It is the most specific test made on the cerebrospinal fluid. A fully positive reaction occurs in all cases of paresis. In tabes the reaction may be only 2 or 3 + and in 1 out of 4 cases the reaction is negative. The Wassermann may, in chronic paresis and tabes be positive on the spinal fluid, while the blood Wassermann reaction which is usually also positive may be negative. In about 1 per cent of tabetic cases the spinal fluid Wassermann is positive, when all other findings in the spinal fluid are negative and despite the lightning pains, there are not enough physical signs to make a clinical diagnosis of tabes.

In the meningo-vascular syphilis which causes headache and cranial nerve palsies in the secondary and tertiary stages of syphilis there is a positive Wassermann reaction in 20 per cent of abnormal fluids, a cell increase being a more frequent finding. This condition responds very favorably to two or three months antiluetic treatment, whereas paresis and tabes is resistant.

Examinations of Occasional Value.—**Colloidal Reaction.**—All the colloidal reactions give practically the same readings. The various methods are colloidal gold, the colloidal benzoin, the gum mastic and the paraffin colloidal test recently suggested by Kafka. They differ only in the substance used to produce the colloid solution and the reaction depends on a protein substance probably globulin. The colloidal benzoin is the most sensitive. The Kafka paraffin colloidal test is very simple and the materials are readily available. It is outlined here.

Preparation of Reagents.—Dissolve 0.3 gm. of paraffin, melting-point 50° to 52° C., in 100 cc. of absolute alcohol heated by immersion into a vessel of hot water. Some of the paraffin will separate out on cooling. Mix the remaining solution with an equal amount of distilled water having a temperature of 50° to 52° C. The result is a turbid colloidal solution which is stable.

The only other solution necessary is 0.3 per cent sodium chloride in distilled water.

Ten small test-tubes holding 3 to 5 cc. and three 1-cc. pipets.

The Test.—In the first of the 10 tubes in a rack place 0.75 cc. of the 0.3 per cent sodium chloride solution and 0.5 cc. in each of the other 9 tubes.

To the first tube containing 0.75 cc. sodium chloride solution add 0.25 cc. of spinal fluid. Mix and transfer 0.5 cc. of the mixture by means of a pipet, to tube No. 2. After mixing by drawing and expelling from the pipet several times, transfer 0.5 cc. of the contents of tube No. 2 to tube No. 3. Mix and transfer 0.5 cc. to tube No. 4. Continue this procedure until tube No. 9 is reached. Discard the 0.5 cc. from tube No. 9 leaving tube No. 10 as a control without any spinal fluid. Add 1 cc. of the paraffin emulsion to each tube and invert to mix the fluids. Let these tubes stand overnight at room temperature.

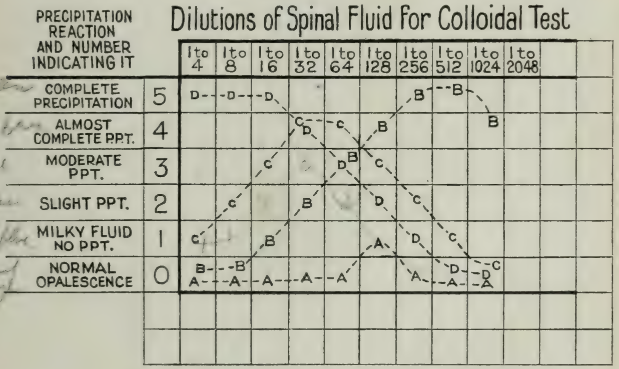

FIG. 68.—Chart of common colloidal curves.

Reaction.—A clearing of the emulsion with a layer of paraffin on top of the fluid indicates a positive reaction and is reported by Fig. 5. No flocculation of the fluid means a negative reaction and is reported as 0. Partial reactions are reported by the figures between 5 and 0.

Interpretation.—No reaction A-A-A- in chart and sometimes reported as 000000000- is normal.

First zone reaction D-D-D- in chart and sometimes reported in figures as 555432100 occurs in syphilitic general paralysis and active disseminated sclerosis. The combination of first zone reaction with a negative Wassermann is evidence in the diagnosis of disseminated sclerosis. First zone reactions also occur in severe toxic delirium and in Mongolism.

Mid-zone reaction C-C-C- in chart and sometimes reported as 123443210, occurs regularly in tabes and meningo-vascular syphilis and occasionally in chronic encephalitis, disseminated sclerosis, brain tumor and senile changes.

End-zone reaction B-B-B- in chart and sometimes reported as 001234554, occurs in acute meningitis.

The chronic diseases which have a relatively small amount of globulin in the spinal fluid produce a reaction in the first tubes which contain a high concentration of spinal fluid. Where the spinal fluid has a large amount of globulin as in acute meningitis, flocculation takes place only in the end-tubes which contain a very minute amount of the spinal fluid.

Many conditions such as old hemorrhages, interference with circulation by tumors or toxic states give a positive protein and colloid reaction so that the reaction is not specific for syphilis as was first thought.

Protein Content.—The best quantitative method is the sulphosalicylic opalescence test as outlined for albumin in the urine. Esbach's picric acid method is not accurate enough when the protein content is slightly above the normal.

Interpretation.—Normal for the spinal fluid is 15 to 45 mgm. per 100 cc. of fluid.

Increase has much the same significance as an increase of globulin but it has the advantage that a definite figure may be recorded.

In syphilitic paresis the protein is around 100 mgm. in encephalitis it is usually below 50 and in brain tumor and cerebral hemorrhage from 100 to 200 mgm. Meningitis shows 200 to 800 mgm. and the subarachnoid block producing the Froin syndrome yields the highest figures which are frequently up to 2000 mgm. per 100 cc.

Sugar Percentage.—*Caution.*—The sugar gradually disappears from the spinal fluid after one-half hour as it does from the blood. The estimation should be made at once or sodium fluoride added.

Method.—Folin-Wu, similar to blood sugar. It is best however to use a double amount of spinal fluid and divide the result by 2. An estimation of the patient's blood sugar should be made simultaneously.

Interpretation.—The normal spinal fluid sugar is 0.04 per cent to 0.07 per cent about one-half of the blood-sugar figure.

Because the blood sugar varies during the day and the spinal fluid sugar is usually one-half the blood sugar it is necessary to have both estimations before attaching importance to any variation in the spinal fluid sugar.

The chief value of the estimation is to differentiate tuberculous meningitis in which the spinal fluid sugar is low, from acute encephalitis in which the spinal fluid sugar is normal or increased. Acute pyogenic meningitis causes a total absence of sugar in the spinal fluid. The fluid sugar usually remains at 45 to 65 per cent of the blood sugar except in meningitis where it is less. Only in diabetes does the fluid sugar go over 0.1 per cent. In coma it may reach 0.2 to 0.3 per cent.

Chlorides.—*Method.*—This may be performed in the same manner as the quantitative chlorides in urine (page 313), although where the test is frequently done other modifications are better.

Interpretation.—Normal 0.72 per cent to 0.75 per cent.

Readings over 0.75 indicate a lack of renal function.

Readings below 0.72 per cent are found in generalized meningitis whereas in poliomyelitis, encephalitis, brain tumor or abscess the readings are normal. As low as 0.5 may be encountered in tuberculous meningitis. In acute pyogenic meningitis the chlorides are not so markedly decreased, rarely going lower than 0.6 per cent.

The blood chlorides are about four-fifths of the spinal fluid chlorides. As this relationship is always maintained, it is necessary to keep in mind the low chloride content in pneumonia.

Urea.—*Method.*—The same as for blood urea nitrogen.

Interpretation.—Normal 10 to 20 mgm. per 100 cc.

Nephritis 20 to 50 mgm. per 100 cc.

Uremia 100 or more mgm. per 100 cc.

The urea nitrogen in the cerebrospinal fluid is the same as that in the blood under all conditions and the estimation of the urea content of one of these fluids is sufficient. Its estimation is frequently valuable in acute disease where renal insufficiency may be the cause of uremic symptoms and coma.

Differential Precipitation Test (Levinson).—Place 1 cc. of spinal fluid in each of 2 small test-tubes. To one add 1 cc. of 1 per cent mercuric chloride solution and to the other 1 cc. of a 3 per cent sulphosalicylic acid solution. Shake well, cork and allow to stand overnight at room temperature.

Interpretation.—If the tube containing mercuric chloride shows two to three times as much precipitate as the one containing the sulphosalicylic acid the meningitis is probably tuberculous rather than pyogenic. This test is sometimes useful when bacteria cannot be discovered or the cells are not diagnostic.

Nonne-Appelt Reaction.—*The Test.*—Mix 3 cc. of cerebrospinal fluid with 3 cc. of saturated ammonium sulphate which is neutral in reaction. Phase I occurs after three minutes when the globulin fraction precipitates. Phase II may be obtained by filtering off the precipitate and boiling the filtrate. Any precipitate which occurs on boiling is a serum albumin.

Interpretation.—Phase I is strongly positive in paresis, tabes and encephalitis whereas Phase II is more marked in all forms of acute meningitis.

THE SPINAL FLUID EXAMINATION IN CHILDREN.

Lumbar Puncture in Children.—The details of lumbar puncture in children are much the same as for adults except that the needle has to penetrate only 2 to 3 cm. (about 1 inch) before reaching the spinal canal in the very young and 3 to 4 cm. in children about ten years of age.

Pressure.—The normal cerebrospinal fluid pressure of infants in the recumbent position is equal to 50 to 100 mm. of water. This is markedly increased by coughing, crying or even extending the head backward. With increased intracranial pressure, there will be a bulging of the anterior fontanelle which remains open to about eighteen months of age.

Cells.—In infants of a few days to a few weeks up to 30 cells may occur normally in the spinal fluid. This cell count gradually decreases until at ten to twelve years it is identical with the spinal fluid of an adult.

Globulin.—Some infants up to several weeks of age show an increased amount of globulin normally. The protein content of the fluid is about normal. All other tests give practically the same reading as in adults.

CEREBROSPINAL FLUID IN DIFFERENTIAL DIAGNOSIS.

Freemont-Smith and Ayer.

Disease.	Initial pressure: mm. of spinal fluid, horizontal position.	Rise on jugular compression.	Appearance.	Cells per c.mm.	Globulin.	Protein, mgm./100 cc.	Sugar, mgm./100 cc.	Chlorides (as NaCl), mgm./100 cc.	Non-protein nitrogen, mgm./100 cc.	Gold sol.	Comment.
Normal Lumbar	70–190	Prompt	Clear Colorless No clot	0–5	0	15–45	50–75	720–750	12–18	0000000000	Sugar and chloride values apply to fasting non-febrile individuals.
Normal **Cisternal**				0–5		10–25	50–75				
Normal **Ventricular**				0–3		5–15	55–80				
Blood plasma	——	——	——	——	——	6,200–8,200	70–110	570–620	18–30	——	
Meningismus	+	N	N	N	0	**N or Low**	N or +	**N or Low**	N	N	**Large amount of fluid with relatively slight drop in pressure.**
INFECTIONS OF THE CENTRAL NERVOUS SYSTEM.											
Tuberculous meningitis	✛	N	Opalescent to turbid Faint yellow ± **Delicate fibrin web**	+ Mononuclears	+	✛	**Low**	**Very Low**	N	Variable	**Excess fluid. Excess polys very early and in infants. Chloride usually below 640 mgm./100 cc. Progressive fall in sugar which may be high at outset, to under 30 mgm./100 cc. or lower. Tubercle bacilli found in clot or sediment, guinea-pig inoculation positive.**
Brain abscess	✛	See brain tumor	Clear and colorless to turbid Clot ±	✛	±	**Slight increase**	**N or ✛**	**N**	N	Variable	**Polys nearly always present.** If complicated by septicemia or high fever chlorides may fall to low levels (brain abscess represents one form of "aseptic meningitis").
Acute syphilitic meningitis	✛	N	Clear to turbid Faint yellow ± Fibrin clot	+	+	+	**N or slightly low**	**Slightly low**	N	**Strong reaction Zone variable**	**Wassermann reaction nearly always positive.**
Acute purulent meningitis	✛	N	**Clear to purulent Faint yellow ✛ Clot ✛ —**	+	+	+	**Low**	**Low**	N	Variable	Sugar may be but slightly decreased at outset. Often falls rapidly to under 10 mgm. Chlorides rarely below 640 unless septicemia is present. Meningococci and influenza bacilli found in smear and culture with difficulty; pneumococci, streptococci and staphylococci easily. Indol present in influenza meningitis.

Acute anterior poliomyelitis	**✚**	N	Slight opalescence Rarely turbid Faint yellow ± Delicate fibrin web ±	+	Slight +	**Slight ✚**	**N or slight increase**	**N or slight decrease**	N	Variable	**In preparalytic stage, polys may exceed 80%—rapid change to mononuclears With gradual decrease in cells, protein increases for two to three weeks.**
Encephalitis lethargica	N	N	N	**N or slight increase No polys**	±	**N or slight increase**	**N or slight increase**	N	N	Variable	**Sugar is normal unless blood sugar is elevated. Over 50% of cases have normal cell count, which rarely exceeds 60 cells. Protein increase when present is slight—rarely reaching 100 mgm./100 cc.**

CENTRAL NERVOUS SYSTEM SYPHILIS.

Acute syphilitic meningitis	+	N	Clear to turbid Faint yellow ± Fibrin clot	+	+	+	**N or slightly low**	**Slightly low**	N	**Strong reaction Zone variable**	**Wassermann reaction nearly always positive.**
"Meningo-vascular syphilis"	±	N Rarely delayed	N Rare fibrin clot	+	+	+	N	N	N	Variable	**Wassermann reaction nearly always positive.**
Progressive parenchymatous syphilis	+	N	N Rare fibrin clot	+	+	+	N	N	N	**Paretic or luetic**	**Wassermann reaction always strongly positive.** (Includes tabes, paresis, and optic atrophy)
Late inactive forms	N	N	N	N	N	±	N	N	N	Weak Luetic ±	**Wassermann reaction weakly positive or negative.**

BRAIN TUMOR.

Above tentorium Not involving wall of ventricle	**Lumbar**	**✚**	**N**	N	N	N or slight increase	**N or slight increase**	N or +	N	N	Variable	Tumors involving optic chiasm may give increased cells (mononuclears). Lumbar puncture in patients with choked disks may be dangerous unless ventricle is tapped at the same time.
	Ventricular	**✚**	**N**	N	N or slight increase	N	**N**	N or +	N	N	N	Ventricular fluid is normal.

CEREBROSPINAL FLUID IN DIFFERENTIAL DIAGNOSIS—Continued.

Freemont-Smith and Ayer.

Disease.			Initial pressure: mm. of spinal fluid, horizontal position.	Rise on jugular compression.	Appearance.	Cells per c.mm.	Globulin.	Protein, mgm./100 cc.	Sugar, mgm./100 cc.	Chlorides (as NaCl), mgm./100 cc.	Non-protein nitrogen, mgm./100 cc.	Gold sol.	Comment.
BRAIN TUMORS - Continued.													
Above Tentorium.	**Involving wall of ventricle**	**Lumbar**	✚	**N**	N occasionally faintly yellow	N or slight increase	+	✚	N or +	N	N	Variable	Protein increase here comes from ventricle.
		Ventricular	✚	**N**	N occasionally faintly yellow	N or slight increase	+	✚	N or +	N	N	Variable	Protein increase occurs in lateral ventricle invaded by tumor—other ventricle may have normal fluid.
Below tentorium	**Intracerebellar and fourth ventricl**	**Lumbar**	✚	**N or delayed**	N	N	Slight increase or N	**Slight increase or N**	N or +	N	N	Variable	Fourth ventricle tumors usually do not cause any protein increase. **The protein increase below intracerebellar gliomas is slight, usually under 100 mgm./100 cc.**
		Ventricular	✚	**N**	N	N	N	**N**	N or +	N	N	N	Ventricular fluid is normal—**Subtentorial block** is best demonstrated by combined ventricular and lumbar puncture.
	Extracerebellar	**Lumbar**	✚	**N or delayed**	N or faintly yellow Rare clot	N	+	✚	N or +	N	N	Variable	**Protein increase in these cases is well marked. Usually 150 mgm./100 cc. or higher.**
		Ventricular	✚	**N**	N	N	N	**N**	N or +	N	N	N	Ventricular fluid is normal.
SPINAL SUBARACHNOID BLOCK (TUMOR, POTT'S DISEASE, MENINGITIS, ETC.).													
Partial block		**Lumbar**	**N or low**	**Delayed**	**N or faint yellow Clot rare**	±	✚	✚	N	N	N	Variable	Partial block is best demonstrated by combined cistern and lumbar puncture.
		Cisternal	**N**	**Prompt**	**N**	±	**N**	**N**	N	N	N	Variable	Lipiodol arrest, but some passes by the block.

Complete block	**Lumbar**	**Low or N**	**Absent**	**Colorless with slight clot, to deep yellow with massive coagulation**	±	++	+++	N	Slightly low	N	Variable	Little fluid obtainable. Rapid drop in pressure to zero.
	Cisternal	**N**	**Prompt**	**N** **Faint yellow color or clot Rare**	±	±	±	N	N	N	Variable	Cistern puncture not necessary to demonstrate block. Lipiodol completely arrested.
Tumor of cauda equina	**Lumbar** (below tumor)	**Low**	**Absent or delayed**	**Colorless with slight clot, to deep yellow with massive coagulation**	N	++	+++	N	——	——	Variable	
	Lumbar (above tumor)	**N**	**Prompt**	Yellow or colorless, clot ±	N	+	++	N	——	——	Variable	
	Cisternal	**N**	**Prompt**	N Yellow color or clot rare	N	±	±	N	——	——	Variable	
Radiculitis of cauda equina also numerous cases of acute polyneuritis		**N**	**N**	**N or yellow Clot ±**	±	+ to ++	+ to +++	N	N or slightly low	N	Variable	Radiculitis of cauda equina difficult to distinguish from tumor, unless puncture is below lesion and block can be excluded. Lipiodol valuable.

MISCELLANEOUS.

"Bloody tap" (normal fluid)		**N**	**N**	**Blood-tinged or bloody supernatant fluid colorless or pink clot**	**No excess of WBC**	±	**Slight increase**	N	N	N	Variable	**Progressively less bloody in consecutive tubes. Supernatant fluid never yellow (at original puncture) even after standing. If much blood present clot will form.**
Early subarachnoid or ventricular hemorrhage (within 24 hrs.)		**+**	**N**	**Bloody + supernatant fluid faintly yellow No clot**	**Slight excess of WBC usually polys Many crenated RBC**	Slight increase	**Slight increase**	N	N	N	Variable	**All tubes equally bloody.** Yellow tint to supernatant or centrifuged fluid appears within 2 to 4 hours after hemorrhage occurs, and deepens.
Late subarachnoid or ventricular hemorrhage (up to two weeks)		±	**N**	**Bloody ± supernatant yellow No clot**	**Excess of mononuclears RBC crenated**	Slight increase	**Slight increase**	N	N	N	Variable	Yellow color will increase for 8 to 12 days, when all RBC usually have disappeared. Compare with Froin syndrome.

CEREBROSPINAL FLUID IN DIFFERENTIAL DIAGNOSIS—Continued.

Freemont-Smith and Ayer.

	Disease.	Initial pressure: mm. of spinal fluid, horizontal position.	Rise on jugular compression.	Appearance.	Cells per c.mm.	Globulin.	Protein, mgm./100 cc.	Sugar, mgm./ 100 cc.	Chlorides (as NaCl) mgm./ 100 cc.	Non-protein nitrogen, mgm./ 100 cc.	Gold sol.	Comment.
	MISCELLANEOUS—Continued.											
	Original "Froin syndrome"	**Low or N**	**Absent**	**Deep yellow Clear, massive coagulation**	+	**++**	**+++**	N	Slightly low	N	Variable	See **"Complete Spinal Subarachnoid Block"**—above. Distinguish from late subarachnoid hemorrhage by high protein content.
Multiple sclerosis	**Clinically active**	**N or low**	**N**	N	**Slight increase rarely over 30**	Slight increase	**Slight increase**	N	N	N	**Strong reaction Paretic or luetic**	A strong paretic or luetic gold sol reaction in presence of negative Wassermann in patient not previously treated for syphilis, is strong evidence for multiple sclerosis.
	Clinically inactive	**N or low**	**N**	N	**N**	N	**N**	N	N	N	N Weak paretic or luetic.	
	Lateral sinus thrombosis	**+**	**Delayed or absent to jug. Comp. on affected side**	N to turbid Slight yellow color or clot rare	Usually slightly increased	N	**N or low if uncomplicated**	Increased or N	**Low or N**	N	Variable	Considerable normal variation on the two sides is unilateral jugular compression in many normal individuals. A prompt response on each side excludes complete lateral sinus thrombosis.
	Uremia	+	N	N	N	N	**N or low if uncomplicated**	+	May be increased or decreased	**Increased**	**Variable—may give strong paretic curve.**	

Methods:
Globulin—Ross, G., and Jones, E.: British Med. Jour., 1909, **1**, 1111.
Protein—Denis, W., and Ayer, J. B.: Arch. Int. Med., 1920, **36**, 436.
Sugar—Folin O., and Wu, H.: Jour. Biol. Chem., 1920, **41**, 367.
Chlorides—Van Slyke, D. D.: Jour. Biol. Chem., 1923, **58**, 523.
Nonprotein nitrogen—Folin O., and Wu, H.: Jour. Biol. Chem., 1919, **38**, 87.

THE CEREBROSPINAL FLUID IN MENTAL AND NERVOUS DISEASES.

It is disappointing that spinal fluid examination is of little aid in the diagnosis of mental diseases. General paresis, which is uncommon, is the only disease giving constant findings. A few suffering from acute mania or dementia precox show a slight excess of globulin. This same lack of information is true regarding many neurological diseases which are progressive. In cerebral atheroma, even when due to syphilis, senile degeneration of the brain, most paraplegias, disseminated sclerosis and encephalitis and brain tumors, the cerebrospinal fluid may be normal. Despite this the detection of increased intracranial pressure, blocking in the lateral sinuses or spinal canal, and the positive knowledge regarding meningeal inflammation makes the examination a highly valuable one.

VALUABLE AIDS IN NEUROLOGICAL DIAGNOSIS.

Eye Examination:

Ophthalmoscopic for: (a) Papilledema of increased intracranial pressure. (b) Optic atrophy of the degeneration seen in neuritis and taboparesis.

Perimeter for: (a) Peripheral nerve disease.
- **(b) Injury to the chiasma in pituitary tumors.**
- **(c) Cortical injury to occipital lobe producing an hemianopic scotomia.**

X-ray:
- **(a) For detection of skull fractures and meningiomas.**
- **(b) Erosion of anterior clinoid processes in cerebral tumors and the posterior clinoid processes in tumors of the posterior fossa.**
- **(c) Lateral displacement of a calcified pineal body by tumor, etc.**

INDICATIONS FOR LUMBAR PUNCTURE.

1. Suspected intracranial hemorrhage from injury or disease.

2. Acute meningeal symptoms—persistent severe headache, stiffness of neck and spine with fever, inability to bring the knees near the chin.

3. Convulsions, coma, hemiplegia and persistent vomiting. (N.B.—Papilledema.)

4. Spinal cord symptoms, spastic paraplegia, palsy of limbs.

CHAPTER VIII.

EXAMINATION OF THE GASTRIC AND DUODENAL CONTENTS.

A SHORT PRACTICAL EXAMINATION FOR GASTRIC CONTENTS.

The optimum time for examination of gastric contents for diagnostic purposes is:

1. On the resting stomach four hours or more after a meal.

2. One to one and one-half hours after a meal when digestion should be at its height.

Compare the results with the chart of normals for that particular time.

Test-meals.

1. The most suitable meal is 8 arrowroot or soda biscuits and 12 ounces of water.

2. A slightly less efficient meal is 2 thin slices of dry toast with crust removed. This should be well chewed and a cup of tea with sugar but without milk may follow.

3. With ordinary meals, meat increases the acidity; fat delays gastric secretion. Large particles of food frequently block the tube before the stomach is empty.

Equipment.

A gastric tube $\frac{3}{8}$ inch outside diameter with mark at 20 inches.

An Ewald bulb.

pH indicator test papers of dimethyl and thymol blue or 1 ounce Töpfer reagent and tenth normal sodium hydroxide and 1-cc. pipet.

PROCEDURE.

Pass the tube to the 20-inch mark and have the patient recline so the contents will syphon out. If this is not convenient use the bulb to aspirate the contents. Both together work well.

EXAMINE THE CONTENTS FOR:

1. Amount:

Normal fasting content $\frac{1}{2}$ to 2 ounces. Normal content one hour after test-meal 2 to 3 ounces. More indicates hypersecretion or retention.

2. Appearance:

Note (1) Flecks of blood or the brown coffee grounds, which is digested blood.

(2) Presence of mucus or muco-pus.

(3) Stage of digestion of food.

3. Free HCl.

Dip a sheet of the indicator paper containing dimethyl into the contents. A red color indicates much free HCl. Match with the scale to ascertain the degree of acidity. If it be below 10 (pH2) dip a thymol blue indicator paper into the contents. Red indicates free HCl present; yellow denotes its absence.

Normal free HCl.—Fasting, 5 to 30 (pH 2.4 to 1.5). One hour after a meal 20 to 60 (pH 1.7 to 1.3).

ANALYSIS OF GASTRIC CONTENTS.

INTERPRETATION IN BRIEF.—Examination of the contents of the resting stomach gives the most information obtained from a single specimen.

In order of importance comes the amount, the appearance and odor and the free hydrochloric acidity.

AMOUNT OF FASTING CONTENT FOUR OR MORE HOURS AFTER A MEAL.—Normal 1 to 3 ounces. Less than normal is of no significance. Over 3 ounces indicates mucus accumulation in a slowly emptying stomach if the contents are clear. If the contents contain food particles four or five hours after a meal there is gastric atony or retention due to reflex spasm from cholecystitis or appendicitis or there may be organic obstruction of the pylorus. Where there is organic disease of the pylorus such as ulcer or cancer there is usually also spasm. From 1 to 2 quarts of secretion and partially digested food may be present. One to one and a half hours after a test-meal there should normally be not more than 3 ounces of content present.

APPEARANCE AND ODOR.—No particles of food should remain in the stomach over five hours. Food remnants remaining in the stomach over twelve hours indicate organic obstruction. Bile staining due to duodenal regurgitation is normal. Small amounts of mucus occur so frequently that little importance can be attached to it. It is usually the postnasal droppings. If mucus is present in large quantities it indicates a catarrhal gastritis. Flecks of blood may be due to suction of the tube. Large amounts diffusely spread come from an ulcer. When fresh or when no acids is present the blood will be a bright red. Acid turns it a dark brown.

ODOR.—A slightly sour odor is normal for gastric contents. Rancid odor of butyric and lactic acid indicates benign fermentation. Putrid, foul odor is found in the copious gastric contents where a sloughing carcinomatous ulcer is present.

FREE HYDROCHLORIC ACID.—Normal 5 to 20 (pH 2.4 to 1.7) frequently. 0 to 30 (pH 3 to 1.5) occasionally.

A meal stimulates acid production and where there is no acid present before a meal it is frequently present one to one and a half hours after. When present before the meal, its amount is usually doubled or trebled one to two hours after.

Variations in the free hydrochloric acid of gastric contents are not in themselves pathognomonic of any disease. Where the symptoms and physical signs point to either gastric ulcer or cholecystitis a low acidity suggests a diagnosis of cholecystitis. A persistently high hydrochloric acid content is suggestive of gastric and especially duodenal ulcer but a normal or low acidity by no means excludes these diagnoses.

In the treatment of gastric ulcer the dose and frequency of alkaline medication should be such that the free hydrochloric is never above 15 (pH 1.8). This determination is best made in the late afternoon.

Free hydrochloric acid is never present in pernicious anemia and but rarely in any anemia. It is absent in over 75 per cent of patients suffering from gastric carcinoma when they seek medical advice.

Gastric analysis is useful in establishing a diagnosis of achlorhydria associated with diarrhea where the administration of dilute hydrochloric acid gives gratifying results.

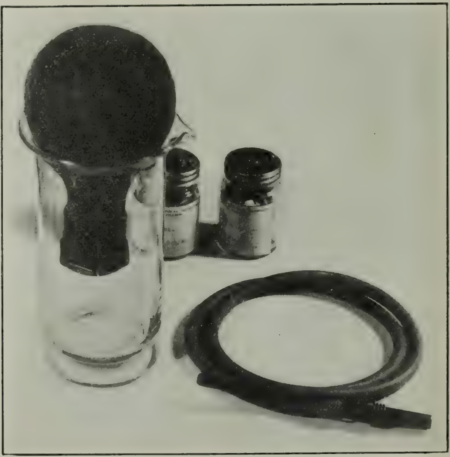

Fig. 69.—Essential equipment for examination of gastric contents. Gastric tube and bulb; 16-ounce measuring glass and indicator papers to estimate the acidity.

EXAMINATION OF GASTRIC CONTENTS IN DETAIL.

Principle.—The gastric contents gives information on gastric secretion and motility by an examination of the chemical constituents and the amount at various times after the taking of food.

Apparatus.—A gastric tube $\frac{1}{4}$ to $\frac{1}{2}$ inch outside diameter and with numerous side perforations near its tip and a mark 20 inches up the tube.

An Ewald bulb extractor.

Two 16-ounce measuring glasses.

Two funnels.

Gauze for filters.

Acid indicator papers containing Töpfer's dimethyl and thymol blue. (Dimethyl indicator (Töpfer's)—Thymol blue 0.1 per cent in alcohol. High-grade filter paper. Pour the dimethyl indicator on a sheet of filter paper and dry as quickly as possible in an air current. Cut into strips 2 inches by $\frac{1}{2}$ inch and place in a wide-mouthed stoppered bottle. Prepare strips saturated with thymol blue in a similar manner and store them in a similar bottle.) (The LaMotte Chemical Products Co., Baltimore, Md., supply these papers with standard colors and directions at a cost of $2.50.)

Or for titration, 1 ounce Töpfer's reagent (0.5 per cent dimethylamido azobenzol in 95 per cent alcohol) in a dropper-bottle and 4 ounces tenth normal sodium hydroxide and 2 1-cc. pipets.

Several large test-tubes.

Procedure in the Single Examination.—Examination of the content of the resting stomach is most important and should be done first. The earliest retention or stasis may be detected by evacuating the stomach four hours after food and examining the content for easily seen food particles such as raisins or prunes. One can later determine by further examination if the retention exists for eight hours or twelve hours. If the fasting content is normal in amount and appearance and if it has 10 to 30 points of free hydrochloric acid no further examination is necessary.

Only when there is no free hydrochloric acid present is it necessary to provide a stimulus in the form of a test-meal and examine the gastric contents one hour after eating. If acid is still absent the examination should be repeated on another day, using a more stimulating meal of meat broth with biscuits and removing the content one and a half hours after the meal when the gastric acidity averages its highest.

The fractional method is so time-consuming that its somewhat inaccurate records of acidity at intervals during digestion does not justify the extra time required for the examination when compared with the application of the single examination as above outlined.

Obtaining the Specimen.—Wash the tube and bulb clean, then immerse them in boiling water for one minute which will sterilize but not destroy the life of the rubber. Assure the patient that the examination is neither painful nor dangerous and that many people have had it previously with only a moderate amount of discomfort and retching. Attach a towel, bib fashion, in front of the patient who should be in the sitting position. Give him in one hand a dish to expectorate into and in the other a large piece of gauze to wipe away mucus. If dental plates are present they should be removed. The operator after washing his hands passes the moistened tube down inch by inch into the patient's stomach. Deep breathing will minimize the tendency to vomit. Encourage the patient to spit out mucus rather than to swallow it. The distance required to reach the stomach is about 20 inches but varies according to the size of the individual. A straight line from the patient's incisor teeth to the anterior-superior spine while he is in the sitting position is about the correct distance to insert a stomach tube. This distance may be marked for each patient by a small adhesive strip on the tube.

There are two methods of removing the contents, one by having the patient recline and expressing the contents through the tube by tightening the abdominal muscles as in retching. The second method is to place slight suction on the tube by applying the deflated Ewald bulb and allowing it to fill. Either way is satisfactory provided the stomach content contains no solid particles. It is best to have the patient recline on the left side and use bulb suction. Move the tube up and down a few inches in case the tip is not in contact with the gastric contents. Shredded wheat, arrowroot, or soda biscuits produce a more uniform liquid content than does slices of bread. Much depends on mastication.

Examination of the Specimen.—The total amount removed is the most important determination to be made on the gastric contents. This is especially true with regard to the fasting content. The presence or absence of mucus is also quite important and when present it gives a stringy character which may be noted in emptying the extractor into the measuring glass or by passing a glass rod through the content and noting if any transparent stringy material adheres to it.

After one hour of digestion of biscuits the gastric contents should be homogenous, of a grayish-buff color and when placed in a container a sediment consisting of chewed and partly digested food particles, settles to the bottom leaving an upper layer of slightly cloudy fluid made up of the water from the meal and the gastric juice.

The presence of blood which is red when fresh and dark brown when acted upon by the acid content for a few moments, should also be noted. Reddish or brown blood diffused throughout the contents indicate pathological gastric bleeding. Small flecks of blood which may adhere to the tube are usually the result of trauma produced by the tube itself. Odor is of importance, the normal content having a slightly sour odor. The pungent odors due to volatile or organic acids suggest a fermentation due to stasis. A foul odor suggests a gastric carcinoma as a carcinomatous ulcer in the stomach is as foul as in any other location in the body.

Test-meals.—It is very confusing to note the large number of test-meals advised by various authors, each one supposed to have special diagnostic values. All test-meals serve much the same purpose, namely to stimulate the gastric secretion in a uniform manner and as nearly normal as possible. The patient's usual diet would be ideal in that it shows his usual gastric activity but large food particles may block the opening in the tube and so only a part of the content may be recovered. As the diets of different people vary greatly. They will in themselves produce variations in the secretory response. Most of the test-meals advised are satisfactory but it is desirable that the material should be readily obtained and should pass easily through the gastric tube. The most satisfactory substances are (1) 8 arrowroot or soda biscuits and 12 ounces of water. (2) A shredded-wheat biscuit and 12 ounces of water. (3) 1 pint of thin gruel strained through coarse gauze. (4) Two slices of dry toast and 8 ounces of tea without milk. Even a meal consisting of 12 ounces of water elicits considerable response.[1] It is important to give on the evening previous to the examination some characteristic food such as a few raisins or prunes. If retained in the stomach overnight they may be readily recognized in the content.

EMPTYING TIME OF THE STOMACH.—Here one has to consider (1) The time elapsed since eating. (2) The character and amount of the meal. (3) The amount recovered.

After Usual Meals.—The normal stomach will empty itself of vegetables, fruit, bread and milk in three hours and meats, fowl and fish in three to four hours. The hypertonic stomach empties in two to three hours, the hypotonic in four to six hours and the atonic in six to eight hours.

When the emptying time of the stomach is unduly short there usually is no free hydrochloric acid in the stomach contents and there may be diarrhea which is remarkably cleared up by giving the patient dilute hydrochloric acid with meals. Chronic gastritis or duodenal ulcer cause a rapid emptying of the stomach although if scar contraction be produced later the emptying time may be retarded. In multiple small gastric ulcers the emptying time is rapid; in chronic peptic ulcer there may be some food in the stomach after six hours. Occasionally a malignant tumor is of the infiltrating type and may prevent the pylorus closing properly. In such a case the meal leaves the stomach in less than one-half hour.

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[1] Rehfuss, Bergeim and Hawk, Jour. Amer. Med. Assn., 1914, **63**, 11.

Food remnants present twelve hours after eating indicate organic pyloric obstruction although rarely, marked atonic dilatation may be a cause.

This may be summarized by stating that delay in emptying may result from: (1) Chronic gastric or duodenal ulcer near the pylorus producing stenosis and if it exists for a time, very marked dilatation of the stomach will result. (2) Carcinoma involving the pylorus. (3) Atony of the stomach walls occurring either by itself or in general visceroptosis. (4) Lesions of the intestine, ileal stasis, peritoneal adhesion.

The irritative early stages in the inflammatory processes produce a rapid emptying of the stomach whereas cicatricial contraction and spasm about the pylorus which occurs later delays the emptying time. Hypertrophy occurs for a while but later atony and extreme dilatation results.

X-ray examination is a less distressing, more certain but more expensive method of examination for the emptying time of the stomach. If barium is retained over five hours a definite retention is present. In addition one may see unusual contractions, a diverticulum due to an ulcer crater or a filling defect due to a fungating carcinoma.

Amount.—Previously any amount up to 200 cc. if clear was regarded as merely hyersecretion but now there is evidence that this is due to mild benign stenosis with spasm. With definite stenosis due to ulcer or carcinoma, vomiting is a valuable symptom. It occurs frequently at first and when dilatation takes place it occurs at longer intervals and the amount may be up to 2 quarts. It is acid with a rancid odor in benign stenosis and foul in malignant stenosis. Examination of the fasting content is valuable in such cases.

Chemical Examination.—Test the acidity either by indicator papers or titration. Filtration is necessary for titration but not for the pH estimation. If free hydrochloric is absent and carcinoma is suspected, the lactic acid test should be performed.

pH Intensity by Reactions on Indicators.—Recently it has been suggested that the hydrogen-ion concentration be estimated by the color change produced on one or two indicators. This is the simplest mode of estimation and it should be employed because we are more concerned with the activity of the acid which is indicated by the pH than its amount which is estimated by titration. We should not be concerned with small differences in pH. It is important to know whether free hydrochloric acid be present and if so whether in small, normal or excessive concentration.

No standard method has been generally adopted. The following methods will give an approximate reading of the hydrogen-ion concentration.

1. One drop of the universal indicator (page 388) added to the unfiltered contents will determine the approximate pH concentration.
2. Acid indicator papers of dimethyl and thymol blue.[1]

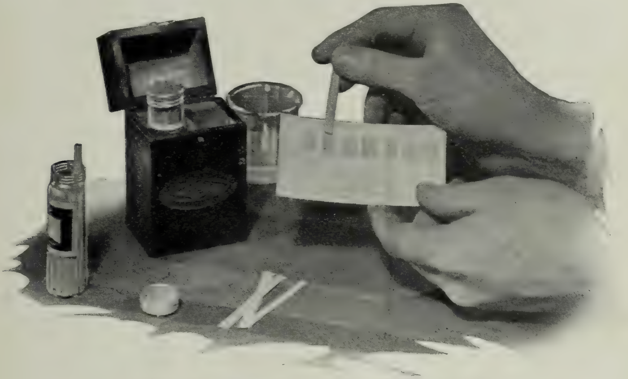

FIG. 70.—LaMotte small gastric acidity outfit.

The Test.—Dip a paper strip containing the Töpfer's reagent in the unfiltered gastric contents and note the color immediately. Deep red indicates a hyperacidity, reddish-orange a normal acidity. If the color is yellowish-orange test with the thymol blue paper and estimate the pH from the color according to the chart below. When the color is deep red mix the content with an equal amount of water. Test the reaction with the paper and divide the result by 2.

Indicator.	pH.	Color.	Equivalent Free HCl.	Remarks.
Töpfer's reagent .	1.4	Deep red	44	Hyperacidity.
Töpfer's reagent .	1.6	Reddish-orange	27	Normal acidity.
Töpfer's reagent .	1.8	Orange	17	Normal acidity.
Töpfer's reagent .	2.0	Yellowish-orange	10	Hypoacidity.
Thymol blue . .	2.0	Purple-red	10	Hypoacidity.
Thymol blue . .	2.4	Faint pink	4	Hypoacidity.
Thymol blue . .	3.0	Yellow	1	Anacidity.

Titration for Free Hydrochloric Acid.—Filter about 10 cc. of gastric contents through fine gauze or muslin. Filtration through filter paper is too slow, especially if mucus is present.

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[1] Denis and Silverman: Am. Jour. Med. Sci., 1925, **169**, 25.

Estimation of Free Hydrochloric Acid.—To 1 cc. of filtered gastric contents in a test-tube add 1 drop of the dimethyl indicator (Töpfer's reagent). If it turns red free hydrochloric acid is present and to estimate its amount add carefully, drop by drop, from a 1-cc. pipet tenth-normal sodium hydroxide until the red color turns a definite yellow. Multiply the quantity of decinormal required by 100 to arrive at the amount of free hydrochloric acid.

Example.—If the color changes from red to yellow after 0.37 cc. of decinormal sodium hydroxide are added then the free hydrochloric acid reading is 100 multiplied by 0.37 or 37.

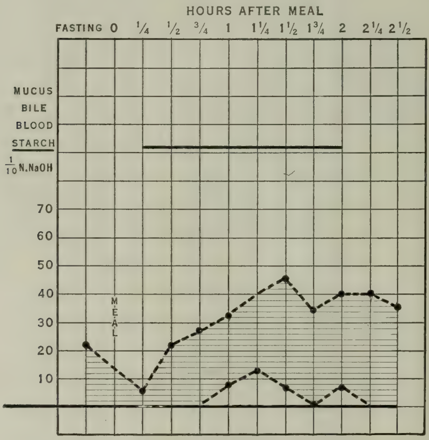

Fig. 71.—Free hydrochloric acidity in gastric contents by the fractional method. Standard chart. The shaded area represents the limits of free hydrochloric acid for 80 out of 100 healthy men. The other 20 show acidities varying outside the shaded area. Four have complete absence of free hydrochloric throughout the test and some of the others show, two hours after the meal, free hydrochloric acid readings up to 100. (Bennett and Ryle.)

Note.—This is the common mode of expressing the quantity of free hydrochloric acid. It means the number of cubic centimeters of tenth-normal sodium hydroxide that would be required to neutralize 100 cc. of filtered gastric contents.

The estimation of total acidity is unnecessary because it is always from 10 to 20 points higher than the free hydrochloric acid. In cases of marked butyric and lactic acid fermentation, there may be 30 to 50 points of a difference between the free hydrochloric acid and the total acidity but the typical "rancid butter" odor of the fermentation and the absence of free hydrochloric acid will enable one to detect this condition without the actual estimation of total acidity.

Interpretation.—Free hydrochloric is present in the normal stomach at all times in the concentration shown on chart, page 266. Its concentration is increased by food, reaching its greatest height from one to one and a half hours after eating. It varies greatly in different individuals and to a less extent in the same individual from day to day. A few have very high free hydrochloric acidity. While this occurs in some apparently normal individuals if it persists, associated with hypertonic contractions and a spastic state of the pylorus it is probably a predisposing cause to gastric ulcer. The spastic or obstructed pylorus prevents the normal regurgitation of alkaline duodenal contents.

It is only when medical treatment is not successful that gastro-enterostomy should be performed for gastric ulcer. A gastro-enterostomy will usually decrease the gastric acidity by 60 per cent.

It is very important in gastric ulcer to arrange the rest, diet, and alkaline treatment so that there is little or no free hydrochloric acid in the stomach at any time. The free hydrochloric acid should never be over 15 in the treatment of gastric ulcer or the pepsin will be activated and erode the ulcer. The symptoms may be relieved with small doses of alkali but large doses may be necessary to keep the free hydrochloric acid below 15. Frequent examinations of gastric contents in the afternoons is therefore essential in the scientific management of gastric ulcer.

Some have no free hydrochloric acid present even after food. This condition is commonly referred to as achylia although strictly speaking this term applied to the absence of both free hydrochloric acid and gastric ferments. If temporary, this condition may be due to psychic causes or some weakening disease. Achylia gastrica frequently exists for many years before pernicious anemia develops. Bacteria multiply and fermentation occurs in the stomach contents when free hydrochloric is absent. Most patients having severe anemias of other types and patients with advanced cancer also have achylia. We may therefore regard achylia as caused by direct irritation of the mucosa or by the blood stream carrying toxins which act on the gastric parenchyma. This produces the anatomical picture of gastritis, a disease of the glandular parenchyma primarily, and secondarily catarrh of the epithelial cells lining the mucosa which ultimately results in atrophy. (Knud Faber.) Achylia is often symptomless but it does not remain so indefinitely. It occurs very frequently and seldom can a definite cause be found. It is present in 40 per cent of working people over fifty years of age. It is usually present in the later months of pregnancy and in children after febrile diseases or gastro-enteritis.

While free hydrochloric is absent in the stomach contents of over 75 per cent of patients suffering from gastric cancer, it is safe to say that not more than 5 per cent of elderly patients who have achlorhydria also have gastric carcinoma. Achlorhydria should therefore be regarded only as a confirmatory sign of such a diagnosis. The finding of a greatly increased amount of gastric content having a very foul odor, and containing lactic acid and Boas-Oppler bacilli and a positive reaction for occult blood in the stool are more reliable signs of gastric carcinoma.

Total Acidity.—To 1 cc. of filtered gastric contents in a test-tube add a drop of phenolphthalein indicator and then from a 1-cc. pipet add carefully drop by drop tenth-normal sodium hydroxide until a pink color develops. Multiply the result by 100 to obtain the total acidity.

Example.—If 0.65 cc. of tenth-normal sodium hydroxide be required to produce the red change, then 0.65 multiplied by 100 equals 65, which is the total acidity expressed in cubic centimeters of tenth-normal sodium hydroxide required to neutralize 100 cc. of gastric contents.

Interpretation.—The total acidity is made up of the free hydrochloric acid together with the hydrochloric acid which has combined with the protein and mucus and the organic acids such as lactic and butyric which result from fermentation. Its estimation is of no practical importance. It is usually from 10 to 20 points higher than free hydrochloric acid except when large amounts of lactic and butyric acid occur as a result of fermentation. Then it may be from 20 to 50 points greater than the free hydrochloric acid. These organic acids when present in large amounts may be detected by their rancid and penetrating odor.

Lactic Acid.—*Reagents.*—Five per cent aqueous solution of ferric chloride.

Eight per cent aqueous solution of mercuric chloride.

Add 2 drops of 5 per cent ferric chloride solution to ½ inch of filtered gastric contents in one tube and also to ½ inch of water in a tube of similar size. To each tube add 6 drops of a saturated solution of mercuric chloride.

If lactic acid be present a deep yellow color is produced in the tube containing the gastric contents in contrast to the other tube which contains only the ferric chloride solution.

Interpretation.—Lactic acid is a sign of stagnation of gastric contents in the absence of hydrochloric acid. It is found in pyloric spasm and obstruction where there is no free hydrochloric acid to inhibit the action of the fermenting lactic acid bacteria. It is usually present in carcinoma after it has advanced to the stage where it is inoperable. Occasionally small amounts may be present however in benign stenosis where there is no free hydrochloric acid.

Blood in the Gastric Contents.—The naked eye examination for blood is more valuable than chemical tests. Diffuse red blood or brown acid hematin diffused through the contents is usually the result of gastric bleeding. Fresh blood flecks are usually from trauma of the tube.

The chemical tests for occult blood in gastric contents are valueless because there is great chance of causing a slight hemorrhage by the use of the gastric tube. If a bleeding ulcer or carcinoma is suspected it is better to test the stool for occult blood as outlined (page 322).

Microscopic Examination of the Gastric Residue.—This examination has a very limited application and need only be done occasionally if carcinoma is suspected. Fasting contents where no free hydrochloric acid is present is best for the test. If food retention is present it can be seen on naked eye examination. Smear a small amount of the gastric contents on a glass slide, fix by heat and stain with Gram's stain and examine under the oil-immersion lens.

The Boas-Oppler are bacilli larger than ordinary forms, having a length two to five times the diameter of a red cell and occurring in jointed chains. They are Gram-positive and stain brown with Lugol's solution while similar organisms from the mouth stain blue.

Interpretation.—Boas-Oppler bacilli are found in stomachs having a retention and no free hydrochloric acid. A carcinomatous ulcer seems to favor their growth as it is in this condition that they are found in the greatest numbers. Bacteriologically they are related to Bacillus bulgaricus and acidophilus. They are of diagnostic significance only when many are present to each microscopic field.

FRACTIONAL GASTRIC ANALYSIS.

Apparatus and Reagents.—Rehfuss tube and large glass syringe.

Ten test-tubes numbered from 1 to 10 in a rack fitted with funnels and muslin filters. Indicator papers for pH or indicators and deci-normal sodium hydroxide and pipets for titration as outlined under examination of gastric contents in detail.

Introducing the Tube.— *Directions to Patient.*—The patient eats a regular supper the previous evening which should include meat, but no food or drink is taken up to the time of the examination at 9 A.M. on the morning of the test.

Obtaining the Specimen.—Sterilize the tube by a minute's boiling. Twenty-two to 24 inches is the average distance from the incisor teeth to the fundus of the stomach. The distance for each person is equal to a line from the incisor teeth to the anterior-superior spine with the patient in the upright position. Mark this length of tube with a small adhesive band. Coil it up and give it to the patient instructing him to swallow the bulb end inch by inch. He will probably gag and retch as the bulb passes by the glottis but this can be overcome by deep breathing. Have him continue to swallow up to the mark showing that the bulb has reached the fundus of the stomach. Then apply the syringe and with gentle suction remove the fasting content.

With the tube *in situ* give the patient a test-meal of biscuits and 2 glasses of water. Fifteen minutes after the meal is finished attach the syringe to the tube, fill and empty it four times so as to obtain a uniform mixture of the contents in the stomach. Then remove 10 cc. and place it on the filter in tube No. 2, the fasting content being in tube No. 1. Blow back a small amount of air through the tube so no gastric content will remain in it to be taken by the next specimen. If the contents cannot be obtained through the syringe instil a syringe full of air into the stomach, then aspirate. Withdraw a sample in a similar manner every fifteen minutes for two and a half or three hours following the meal. If the stomach is not empty at the end of three hours withdraw all contents and measure its quantity.

Add to each funnel a small drop of Töpfer's dimethyl reagent. A yellow color indicates no free hydrochloric acid. A red color indicates its presence and its concentration should be estimated by one of the methods outlined under "Chemical Analysis of Gastric Contents" (page 264). If no free hydrochloric acid is present in any of the specimens the fasting content should be examined for lactic acid. Chart the results and compare with the normal chart on page 266.

The Large Gastric Tube With Ewald Bulb Compared With the Small Rehfuss Tube.—The small Rehfuss tube causes very little discomfort to the patient. It is necessary for the repeated fractional gastric analysis. Very few cannot swallow it. It is asserted that because the tip rests by gravity on the floor of the stomach it will empty the stomach more completely than the large tube. While this may be true of patients who have no mucus in their stomach and after finely pulverized test-meals I have seen very small amounts of fasting content removed from the stomach by the small tube and on withdrawing it, thick masses of mucus were found plugging the tip. A large tube was then inserted and a pint of fluid containing mucus and food particles removed.

It is where retention or mucus is present in large amounts that it is important to know the amount of the fasting content and it is in such cases that the large tube is more successful than the small one.

The large semi-rigid tube causes considerable gagging and distress because of its size. If its insertion and the withdrawal of contents is carried out expeditiously the whole procedure need not occupy over two minutes. This dispatch compensates for the discomfort. It can be passed on patients who have not the will power to swallow the small flexible tube.

Comparative Values of the Single and Fractional Examination.—The fractional estimation is useful in detecting hydrochloric acid where the acid secretion is delayed beyond an hour. The acidity obtained depends largely on whether the tip rests on an acid-secreting area of mucosa. The hydrochloric acid is secreted from the parietal cells present in the mucosa of the fundus of the stomach, but not in the cardiac or pyloric areas. Many unduly high figures are obtained in this manner and at times some high acidities are not detected when the tube happens to rest on the non-acid secreting area of mucosa at the cardiac end.

I have seen the free hydrochloric acid on the first 10 cc. removed register at 30 whereas the free hydrochloric acid of a mixed sample from the total content removed immediately after would be 50. Comparative estimations usually tally more closely however. Withdrawing and injecting a few syringefuls helps to mix the gastric content and so make the estimations on 10 cc. more representative of the whole.

The greatest contrast between the fractional and single examination is in the time required for the estimations. It requires two and a half hours to three hours to complete a fractional examination whereas obtaining and examining a single specimen by simple methods requires about fifteen minutes and is satisfactory as a routine test if hydrochloric acid is present.

Newer Methods for Investigation of the Gastric Secretion.—The absorption of gastric secretion by a test-meal as well as the patient's fancy for the food are variable factors which vitiate results of analysis of stomach contents. The amount of secretion of pure gastric juice and the response of the hydrochloric acidity after the hypodermic injection of histamine give hope for more uniform results.[1]

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[1] Bloomfield and Polland: The Diagnostic Value of Studies of Gastric Secretion, Jour. Am. Med. Assn., 1929, **92**, 1508.

Technique.—The patient is examined in bed under basal conditions after a twelve-hour fast.

The patient swallows a duodenal tube until it reaches the fundus of the stomach and he spits out any saliva secreted. The fasting contents are withdrawn by a 50-cc. Luer syringe, after which the gastric contents are withdrawn two or three times at ten-minute intervals. Histamine is then injected hypodermically 1 mgm. per 10 kilograms of body weight. Use a solution of 10 mgms per cc. The gastric contents are withdrawn at ten-minute intervals for half an hour to one hour.

The secretion is clear and suitable for chemical estimations.

Measure and record the amount and acidity of each lot aspirated at each ten-minute interval.

Normal.—The maximum ten-minute secretory volume is not under 35 cc. or over 150 cc. and its total acidity is not under 90 and not over 125.

Interpretation of Abnormal Results.—A high acid concentration and large secretory volume occurs in the presence of gastric and especially duodenal ulcers. These findings do not occur in carcinoma. A low acidity and small volume may be taken as evidence against the presence of ulcer and in favor of cancer or severe anemia. High or low acidities and volumes cannot in themselves be regarded signs of disease as they may occur in healthy individuals.

DUODENAL DRAINAGE.

Duodenal drainage is not used as frequently as it was a decade ago. The examination requires two or three hours and it is difficult to be certain whether inflammatory products found, come from the biliary tract, the duodenum, the stomach or the mouth. Thorough gastric lavage and examination of the gastric washing as a control is of value in excluding the stomach or mouth as a source of pus cells and bacteria. Several authentic cases are on record where typhoid carries showed Bacillus typhosus in their duodenal drainage although none could be cultured from the stool. Cholecystectomy proved that cholecystitis was present and typhoid bacilli were cultured from the mucosa.

Obtaining the Specimen.—The patient should eat no food for at least six hours previous to the examination. Pass the sterilized Rehfuss tube and remove any gastric contents present. Wash the stomach with 10 ounces of tap-water three or four times. Save the first washing to compare its sediment with the duodenal contents. Then place the patient on his right side, give him a small glass of water and have him swallow the tube to the 30-inch mark. Instruct him to spit out all saliva. The tube should enter the duodenum in twenty to forty minutes. If the end of the tube hang over the edge of the couch the duodenal contents will syphon into a bottle below.

A slightly viscid bile-stained fluid which may occasionally be slightly acid but usually is alkaline pours out when the tube passes into the duodenum. If 20 cc. of a colored fruit juice be injected it can only be withdrawn if the tube tip is in the stomach. The fluoroscope is a certain method when it is avilable. Wash the duodenum with 8 to 10 ounces of water. To stimulate the bile flow inject slowly through the tube a cube of bouillon in water at the temperature of a hot drink, or an ounce of 0.5 per cent hydrochloric acid, taking three to five minutes for the injection. Thirty-three per cent magnesium sulphate was originally used for this.

Have the bottles labelled *A*, *B*, and *C*. In the *A* bottle collect the first bile which is a light golden-yellow. It is said to come from the duodenum. When the bile comes away from the tube a dark green, collect it in the container marked *B*. This is said to come from the gall bladder or if the gall bladder be removed, from the dilated ducts. It is 30 to 100 cc. in amount. When the outpouting bile comes away a light yellow fluid again collect it in the *C* container. This is said to be liver secretion.

Examination.—The gross examination is valuable. A clear fluid is very favorable evidence that it is normal. A cloudy fluid may be due to inflammatory cells in the bile or it may be due to gastric contents coming down the duodenum.

Centrifuge the fluid for ten minutes and examine the sediment microscopically at once as the cells soon become digested by the fluid. Rub some of it between the fingers to feel for gritty particles. Place some of the sediment on a slide, cover with a cover-glass and examine under the high-power lens for pus cells, bile-stained columnar epithelium, mucus, bacteria and crystals. Compare the findings with the microscopic appearance of the sediment from the gastric wash.

Pus cells, crystals and bacteria in the bile suggest cholecystitis. In the acute cholecystitis, many bacteria are present and frequently growth on media shows a pure culture of Bacillus coli, Bacillus typhosus, staphylococci or streptococci. In patients past middle life who suffer from urticaria or eczema, vaccine made from organisms obtained by a duodenal drainage is under trial.

CHAPTER IX.

EXAMINATION OF THE URINE.

URINE ANALYSIS.

An Outline of the Routine Examination.

The urine should be fresh. The following order of examination is the most time-saving:

1. If there are more than one, number all specimens and containers.

2. Mix well and centrifuge a test-tube full for at least four minutes.

3. **Sugar Test.**—To 5 cc. of Benedict's solution (about 1 inch in a test-tube of $\frac{3}{4}$ inch diameter) add 0.5 cc. (8 drops, not more) of urine. Heat over a flame for one to two minutes, shaking the tube to prevent solution spurting out, or better, stand the tube in boiling water two to three minutes, not more.

Negative.—Clear blue or bluish-green with gray precipitate.

Positive.—Opaque with a yellow or red sediment. The amount of sediment depends upon the percentage of sugar present. If the result is doubtful, allow to cool spontaneously before reading. If the sugar test is positive, test for ketone bodies by the nitroprusside and ferric chloride reactions.

4. Albumin Test.—Fill a half-inch test-tube to the 2.5-cc. mark with clear urine and to the 10-cc. mark with 3 per cent sulphosalicylic acid. Mix. A white turbidity indicates albumin. Small amounts produce an opalescence, large amounts an opaque cloud. For quantitative estimation compare with standards.

5. Record.—(a) Hour passed.
   - (b) Color.—Dark yellow, light yellow, watery, etc.
   - (c) Clearness or turbidity.
   - (d) Reaction.—Use blue litmus. Acid renders it red; alkali, blue.
   - (e) Specific Gravity.—See that the urinometer floats freely. Make correction if at body temperature.

6. Microscopic Examination.—Examine a well-mixed drop of sediment under a cover-glass, using the low-power lens with subdued light. Look for:
   - (a) Pus cells.
   - (b) Red blood cells.
   - (c) Casts.

Disregard cylindroids, mucus, epithelial cells, bacteria, crystals, amorphous sediment and extraneous matter. Less than 50 cells per low-power field or less than 3 perfect finger-shaped casts in the whole sediment is of no significance.

Cells.—If more than 50 cells are present under the low-power field there will be over 3 per high-power field. Differentiate the pus cells from the red blood cells under the high-power lens reporting the approximate number per high-power field after examining 10 microscopic fields and noting if the pus cells are clumped. Record over 100 cells as "much pus or much blood."

Casts.—Use the low-power lens and if more than 3 typical casts are present state the approximate number seen in the whole sediment reporting over 100 as "numerous." They do not have tapering ends or tails as cylindroids do. When more than half the cast is clear it is reported as hyaline; when more than half is granular it is reported as granular. Pus casts, blood casts or epithelial casts are reported as such. The high-power lens may be necessary to distinguish the cells on them.

7. Record findings.

URINE ANALYSIS IN DETAIL.

Reagents and Apparatus.

Benedict's Copper Sulphate Solution.[1]

Sulphosalicylic acid 3 per cent.[2]

Twelve test-tubes ¾ inch by 6 inches; 1-cc. pipet with rubber attached nipple. Twelve centrifuge tubes 15 cc. each; 12 glass slides; 24 cover-glasses No. 3, ⅞ inch square.

Test-tube rack and litmus paper, china marking pencil. Test-tube brush.

Small water-bath. Alcohol burner or other source of heat.

Urinometer graduated 1.000 to 1.040 with long stem.

Microscope.

Centrifuge, turning 1000 to 2000 revolutions per minute. The electrical ones are best.

Collection of the Specimen.—Three to four ounces of fresh urine should be submitted for the test. The morning urine is least likely to show signs of disease. Specimens from females may show much epithelium and a few pus cells from vaginal discharge if it is not washed or douched away before the specimen is passed.

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[1] Benedict's Copper Sulphate Solution:

Copper sulphate (pure crystallized)	17.3 gm.
Sodium or potassium citrate	173.0 gm.
Sodium carbonate (crystallized)	200.0 gm.
or 100 gm. of anhydrous sodium carbonate	
Distilled water to	1000.0 cc.

Dissolve the copper sulphate in 100 cc. of distilled water with the aid of heat. Dissolve the sodium citrate and carbonate in 700 cc. of distilled water, heating if necessary. Then pour in the copper sulphate solution, rinsing the container twice with about 25 cc. of distilled water. When cool dilute up to 1 liter with distilled water.

[2] Thirty grams to 1 liter of water.

Preservation.—If the urine cannot be examined when passed and a cool place is not available, a preservative should be added. There is no ideal urinary preservative. A few drops of toluene is probably the best available. It floats on the surface of the urine which should be pipeted from beneath it for examination. Droplets of it may resemble red blood cells on microscopic examination. Other preservatives in common use are: boric acid, 5 grains to each 4 ounces of urine; formalin 1 drop to each ounce of urine. Formalin if in excess, interferes with the Benedict sugar reaction. Even small amounts give a positive Fehling test. It causes a dense precipitate which makes the microscopic examination difficult. Special tablets of compound substances such as urotropin and benzoic acid are used by insurance companies and they interfere but little with the examination of the specimens.

Clearing the Urine.—Filtration through paper of moderate mesh is the best method of clearing urine. This will remove all turbidity except bacteria, which are only partly removed. Centrifuging will produce the same result as filtering. If the urine is still too cloudy add a small amount of talcum, calcium or light magnesium carbonate to the urine and filter, discarding the first few drops that come through. The powder forms a very fine layer over the filter paper and the bacteria cling to the particles while little or no albumin is lost. Profuse bacterial growth occurs most frequently when urines have stood at room temperature for more than four hours. If the turbidity be due only to urates, warming will cause it to disappear; if it be due to phosphates a few drops of any weak acid will clear it.

Perform the examinations in the following order:

1. Heat the water-bath for the sugar test.

2. If there is more than one specimen, number with a china marking pencil, each label with the patient's name and the bottle containing the urine. Then number every tube or slide in which the specimens are being examined with the same number that is on the name label.

3. Shake the specimen well to mix any sediment, fill a 15-cc. centrifuge tube and centrifuge it for four to five minutes.

4. Add 0.5 cc. (8 drops, not more) of urine to 5 cc. of Benedict's solution and when the water is boiling immerse the tube for two to three minutes (not more, use a watch), after which allow it to cool spontaneously and examine it last.

5. Record the odor, color, clearness or turbidity, reaction and specificity gravity.

6. Perform the albumin test and let it stand while examining the sediment microscopically.

7. Record all the findings. In following this routine there is no loss of time.

SUGAR TEST WITH BENEDICT'S REAGENT.

As it is difficult to pour a few drops from a bottle, a 1-cc. pipet with a nipple placed on the end is not only accurate but is a very rapid means of obtaining the correct amount. As a substitute 10 drops from a medicine dropper may be used (see page 400 for size of drop). It should be rinsed after use.

For a single test it is often more convenient to boil the test-tube over a flame without the water-bath. If this method is used, it is wise to point the test-tube at a paper shield to catch any solution that may spurt out, and to use a test-tube holder.

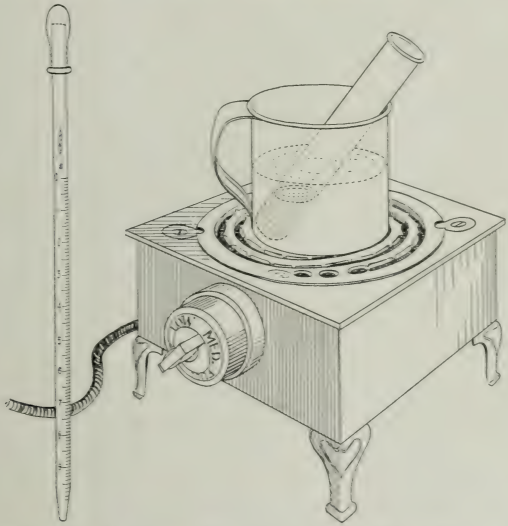

FIG. 72.—The test for sugar, without a flame. Five cubic centimeters of Benedict's solution will fill 1 inch of test-tube $\frac{3}{4}$ inch in diameter. A 1-cc. pipet with a dropper nipple pushed well down to take up exactly $\frac{1}{2}$ cc. is the best way to add the urine. Shake and immerse in water already boiling for two to three minutes. For interpretation of reading see page 278.

(*a*) Add 0.5 cc. (8 drops, not more) of urine to 5 cc. of Benedict's solution in a test-tube (about an inch in a test-tube $\frac{3}{4}$ inch in diameter). Shake to mix the solutions.

(*b*) Heat for one to two minutes, shaking the tube to prevent solution spurting out. (Standing the test-tube in a vessel of boiling water for from two to three minutes, not more, will prevent bumping and is equivalent to the naked flame for one to two minutes.)

(*c*) Allow to stand a few minutes before examining.

Negative.—Transparent blue, occasionally transparent greenish-blue with gray precipitate due to urates.

Positive.—Opaque with a yellow or red sediment. The amount of sediment depends on the percentage of sugar present.

Example.—Green turbidity with slight yellow precipitate at the bottom indicates 0.1 to 0.25 per cent sugar. Yellow or red precipitate which when it settles leaves a clear supernatant fluid, indicates over 1.5 per cent sugar.

If the sugar test be positive, test for ketone bodies by the sodium nitroprusside and ferric chloride tests.

The copper reduction tests are not specific enough to be ideal sugar tests. The best results from simple reactions at present available are obtained by following the technique here outlined for Benedict's test.

The amount of Benedict's solution and especially the amount of urine and the time of boiling should be rigidly adhered to. An excess of urine or boiling for too long will produce questionable reactions in concentrated normal urines. The water-bath method is very satisfactory and avoids the danger of the glass breaking over the naked flame or the contents of the tube spurting out to stain the walls. If immersed in boiling water for over three minutes false reactions will occur in the highly concentrated specimens containing much urate or phosphate. There are many other simple tests for sugar but they have more fallacies than the Benedict test. The Fehling test is the best known of the older ones but it is not only easily reduced by many preservatives but also by a number of drugs the patient may take. Fewer fallacies will occur if equal parts of Fehling's solution and the urine be brought to the boiling point in separate test-tubes, then mixed and no further heat applied. It is better to find out the maximum diet on which the patient can keep a sugar-free urine, than to use a multiplicity of sugar tests.

Interpretation.—Large amounts of sugar constantly present in the urine indicate diabetes; small amounts appearing occasionally may be due to renal or alimentary glycosuria. The sugar tolerance may be tested by giving the patient 100 gm. of glucose or cane-sugar and testing the urine at two-hour intervals for the percentage of sugar. The severity of the diabetes is usually proportional to the sugar present in the urine and its persistence on restricting the diet. In some cases a morning blood sugar is valuable. If a patient who has had a diabetes of long standing becomes careless in the diet or contracts a bacterial infection the blood-sugar percentage may be higher than the urine sugar percentage would indicate. This also occurs frequently in patients who have chronic nephritis. Except in such cases the treatment of diabetes is satisfactory when the urine is kept sugar free. The urine of the diabetic should be tested for acetone bodies.

The Diagnosis of Diabetes as Outlined by Allen in the Oxford Medicine.[1]—"The individual who can eat two full meals consecutively and show a urine free from sugar after each is a safe risk for an insurance company.

"The meals should have 100 to 150 gm. of carbohydrate, 25 gm. of protein, 25 gm. of fat which would be contained in a moderate amount of fish or meat, a medium-sized potato, a portion of rice or macaroni, dessert of apple pie or ice cream, coffee and two lumps of sugar.

"Collect a specimen of urine two hours and four hours after each meal. The specimens after the last meal may be the ones to show sugar. The malicious, by prearranged diet, can take one meal and show no sugar in the urine. Should an exception be found to this statement, the patient would still be a good risk because his diabetes would be mild and he would be so skilful in controlling it that his expectation would be far higher than the ordinary person. This method of determining the presence or absence of diabetes is therefore recommended."

This is perhaps the most applicable means at out disposal. There is no method of detecting diabetes if the patient secretly takes insulin.

Glycosuria During Pregnancy.—A glycosuria occurs in 5 per cent of cases. There is a drop of the renal threshold and a slight elevation of the sugar-tolerance curve which cannot be called diabetic. After pregnancy the glycosuria disappears due to a rise in the renal threshold. The state is of slight significance as the patients can continue with a normal diet, and they have a normal pregnancy and offspring.[2]

ALBUMIN DETERMINATION. SULPHOSALICYLIC METHOD.

Qualitative and Quantitative.—(Standards for quantitative albumin, Kinsbury-Clark type, manufactured by the Fales Chemical Company, Inc., Cornwall Landing, New York.)

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[1] Courtesy of the Oxford University Press, New York.

[2] Williams and Wills: Quart. Jour. Med., 1929, **87**, 493.

The Test.—Urine cloudy from urates or phosphates does not interfere with the test. They clear up on adding the sulphosalicylic acid. Bacteria or much epithelium will in themselves produce an opalescence. One can either filter such specimens or use a control test adding to the 2.5 cc. of urine, water instead of sulphosalicylic acid.

To 2.5 cc. of urine in the special test-tube add 3 per cent sulphosalicylic acid[1] to the 10-cc. mark. Invert the tube to mix. Over 0.02 per. cent albumin produces a white cloud immediately. It becomes more intense on standing for a few minutes. Large amounts of albumin give an opaque white cloud.

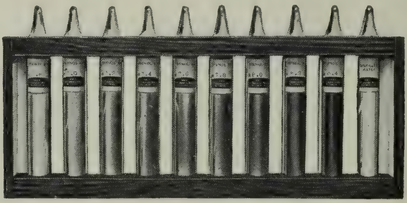

FIG. 73.—Kinsbury-Clark standards for albumin. For the rapid quantitative determination of 0.01 per cent to 0.10 per cent albumin in urine by the sulphosalicylic acid method.

For quantitative estimation allow to stand ten minutes and compare the turbidity with the permanent turbidity standards. Record the value of the standard most closely matched, as the albumin content of the urine. Seven standards are used, equivalent to 0.01 per cent, 0.02 per cent, 0.03 per cent, 0.04 per cent, 0.05 per cent, 0.075 per cent and 0.1 per cent of albumin.[2] In case the specimen contains more than 0.1 per cent of albumin it may be diluted 1 in 10 and redetermined. By multiplying the value then found by 10 the result is obtained.

Heat and Acid Test for Albumin.—(*a*) To a thin-walled test-tube two-thirds full of clear urine add one-sixth of its volume of 35 per cent sodium chloride solution and about 0.5 cc. (8 drops) of 50 per cent acetic acid. Mix by inverting the tube.

(*b*) Holding the tube by its lower end, boil the upper third of the fluid.

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[1] 30 gm. dissolved and diluted to 1000 cc. with distilled water.

[2] Jour. Lab. Clin. Med., 1926, **11**, 891.

(*c*) A white cloud in the heated area denotes albumin. The column of urine below that which has been boiled serves as a control and the cloud is best detected by holding the tube against a black background. Report a faint cloud as a small amount and an opaque cloud as a large amount of albumin.

Boiling the urine and adding a small amount of any acid either before or after the boiling will show albumin in all but a very few cases. The acid is necessary to redissolve the phosphates which are precipitated out on account of the carbon dioxide which hold the phosphates in solution being driven out of the fluid by the heat. A great excess of acid will redissolve the albumin.

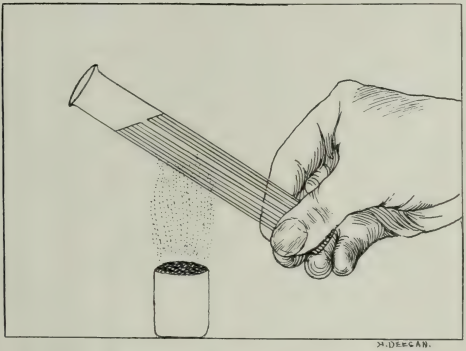

Fig. 74.—Testing for albumin by means of heat and acid.

When the salt content is low as in urines with a specific gravity under 1.010, small amounts of albumin will be missed if extra salt is not added. This dilute urine is usually present in chronic interstitial nephritis with hypertension which is the very type of case where it is important not to miss the albumin. The salt will prevent the precipitation of mucin which may ordinarily be mistaken for albumin. For these reasons, addition of salt solution is advised as a routine. Sodium chloride is advised because it is always available but for those who wish to employ this test as routine, 1 cc. of an acid buffer solution[1] added to each 5 cc. of urine before boiling gives excellent results and is free from fallacies.

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[1] Sodium acetate, 20 gm.; acetic acid, glacial, 44 cc.; distilled water to 100 cc.: personal communication from Dr. F. B. Kinsbury.

Heller's Ring Test for Albumin.—Place 2 or 3 cc. of concentrated nitric acid in a test-tube and carefully overlay it with urine from a pipet so as not to mix the fluids. An opaque white ring at the line of contact of the two fluids denotes albumin. The breadth of the ring depends on the amount of albumin present. When very small amounts are present it may require a minute or two for the ring to appear and it must be examined against a black background to detect it.

Comparative Utility of the Sulphosalicylic, the Heat and Acid and Heller's Cold Nitric Tests.—Any of these methods will detect albumin quite satisfactorily for clinical purposes. The points in favor of the sulphosalicylic acid test is that it is easily performed and as the solutions come together in definite amounts, the degree of turbidity indicates the amount of albumin. A quantitative result can be recorded almost immediately by comparing any turbidity present with the standards. The reagent is inexpensive and is not dangerous to handle. The reaction does not require heat. This method has been adopted by the Committee on Urinary Impairments of the Association of Life Insurance Directors of America. This is a good recommendation from an authoritative source. The chief objection to the sulphosalicylic method of albumin determination is that it is relatively new. If filtering precautions are not taken bacterial growth in stale urines may give 0.01 per cent reaction for albumin by this method. As less than 0.01 per cent of albumin by this test is of no clinical significance, the overcautious may attach importance to very faint reactions if they have not a set of standards. The standards at present cost $13.50 and are only guaranteed against change for eight months although they remain good for a much longer time. Some practitioners carry a small vial of the sulphosalicylic powder and as a bedside test add a pinch to a test-tube of urine. It is not caustic to handle. A white turbidity indicates albumin but this method is rather sensitive.

In the heat and acetic test the reagent is a common one and the test is well known. It has no fallacies if the urine is faintly acid and when the specific gravity is low, extra salt added. If these precautions are not observed fallacious reactions will occur. One frequently sees the test carried out with faulty technique. A naked flame is not always available.

These two tests are equally sensitive and are about four times as sensitive as the Heller ring test. The Heller ring test however has proven satisfactory enough to remain in use for many years. The main fallacy is that occasionally with concentrated urines a hazy ring due to urates but which may be mistaken for albumin appears *above* the juncture of the two fluids. A false ring may occur at the junction of the two fluids due to resinous drugs such as copaiba and tolu balsam, or thymol preservative. Concentrated nitric acid is dangerous on account of its caustic property.

Interpretation.—Albumin is a finding that requires further investigation. If it be due to blood, suspect in the following order: nephritis, a calculus, a neoplasm or tuberculous kidney. If nephritis be a cause casts will be present. One should then determine the renal function and investigate whether heart failure be a cause. Albumin may be due to the febrile diseases and usually disappears when the fever does. Anemias and other blood diseases may be a cause. Albumin in the young may be functional. If so it is diminished or disappears on rest in bed.

In adults where there is an organic lesion of the kidney the largest amounts of albumin are due to tubular degenerations of the kidney. There is no elevation of blood-pressure and the prognosis is usually good. Toxemias of pregnancy with albuminuria and no increase in the blood urea is of the eclamptic type rather than the nephritis. Little or no albumin may be present in arteriosclerotic kidney producing vascular hypertension and a marked degree of nitrogenous retention. The water tests and the blood urea will indicate the seriousness of the lesion in such cases. A constant albuminuria is of more serious significance than an intermittent one.

Albuminuria is of serious significance when associated with increased arterial tension, albuminuric retinitis or when granular casts and red blood cells are in the urine. The history of the case will determine the nature of the disease.

The functional tests are frequently normal when albumin and even casts are present in the urine. Man has more than twice as much kidney tissue as he requires and nephritis must first destroy all the reserve structure before it will interfere with the patient's sense of well-being or show a diminished kidney function by tests of renal efficiency.

The tendency of nephritis is progressive, sometimes very slowly, other times more rapidly and the connective-tissue growth in the healing process destroys the functioning glomeruli and tubules so that in time all the reserve kidney substance is destroyed and the amount needed when the body is at rest is encroached upon. Functional tests will now show impaired renal efficiency.

The significance of albumin and casts in the urine pointing to a progressive lesion of the kidney is borne out by statistics which show insurable persons having constantly a faint trace of albumin at the age of 40 years or over have a death-rate from nephritis which is ten times the normal; with a small amount of albumin (0.01 to 0.02 per cent) the death-rate from nephritis is about thirty times the normal; with large amounts of albumin (0.1 to 0.2 per cent) and casts the death-rate from nephritis is about fifty times the normal.[1]

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[1] Dublin, L.: Am. Jour. Hygiene, 1921, **1**, 301.

The average life expectancy of such a case which should be normally 27.4 more years, is only twelve years.

Physical Properties.—These can be detected very quickly and are often of value in diagnosis. They should therefore be thoroughly understood.

Odor, color, clearness or turbidity, the presence of shreds, sediment and its color, should be noted.

Odor.—Urine normally has a faint characteristic odor. The strong fetid, ammoniacal odor from some specimens is due to bacterial growth which occurs on long standing of the specimen. This may be due to either retention in the urinary bladder as in prostatic hypertrophy or to the urine standing in the specimen bottle twenty-four hours or more before examination. In the latter case a fresh specimen should be obtained where possible. A faint apple blossom odor in urine occurs in severe acidosis present in diabetes or high fevers. It is caused by the presence of large amounts of the volatile acetone.

Hour Passed and Color.—A specimen passed at any hour of the day is suitable for examination. The morning specimen is best to test the concentrating function of the kidney. In mild diabetes the morning specimen is usually free from sugar but an afternoon or evening specimen will show sugar. In examining for pus from a urethritis it is important to know the time since the previous specimen was passed because little or no pus may be present if the urine has been passed within the previous half hour.

The morning specimen is normally highly concentrated, of a deep yellow color and has a high specific gravity. Excessive perspiration will also produce this effect. One should find out the cause of a pale morning urine. About an hour after a meal or after drinking large quantities of water the urine is very pale and has a low specific gravity. The urine of a patient suffering from diabetes and chronic nephritis is also pale so it is important to inquire about the previous drinking of water. In febrile conditions the urine is highly colored, due to the water-loss by perspiration. Smoky red or brown color is usually due to the presence of large amounts of blood. Bile gives urine a brownish or greenish-brown color and a syrupy consistency. Certain drugs alter the color, notably methylene blue which is contained in "Gin Pills" and turns the urine a greenish blue. Phenolphthalein, a common laxative will make the urine, when alkaline, a pinkish-red. Other drugs such as santonin, rhubarb, senna and cascara produce a light brown urine.

Clearness or Turbidity.—Almost all the specimens of normal urine are crystal clear when passed. Because a urine is clear is no guarantee that it is normal, however. It may show albumin, sugar, or a few casts and be quite transparent.

Turbidity, on the other hand is present in most diseased urines but may be present in perfectly normal ones also. A turbidity may be present in a fresh specimen of normal urine passed several hours after a heavy meal. This is due to phosphates or urates.

Usually after a clear normal urine has stood for several hours a hazy sediment crystallizes around a mass of mucus threads that lie at the bottom of the bottle. This is more marked in women on account of the vaginal epithelium and mucus.

Macroscopic blood imparts a smoky brown color to urine. It settles slowly producing a reddish or brown sediment which can only be distinguished from urates or uric acid crystals by microscopic examination. A pinkish-red sediment, which is at time larger in amount, is due to amorphous urate. A white turbidity or sediment is usually due to amorphous phosphates or pus cells.

If the urine remains turbid it is usually due to bacteria. These multiply greatly when the urine has stood for some time and usually are accompanied by phosphatic deposit and ammoniacal odor. A turbidity caused by bacteria, epithelium and pus is a common finding in some female specimens where the urinary tract is normal but infection of the cervix uteri is present.

A Cloudy Urine may be Due To:
Phosphates—not pathological, disappears on adding acid.
Urates—not pathological, disappears on heating.
Mucus—not pathological in moderate quantities.
Bacteria—not pathological if urine has stood several hours, pathological in fresh urines. Does not clear on ordinary centrifuging.
Pus—always pathological.
Blood—always pathological.
Most cloudy urines are not pathological. A microscopic examination will decide.

In the male the finding of white shreds or "floaters" indicates a localized inflammatory lesion of the urethra. These are most often found in cases of chronic urethritis or gleet following gonorrheal infection but may occur in non-gonorrheal urethritis. If these shreds are removed with a platinum loop, placed on a glass slide and when stained are found to contain pus cells and intracellular diplococci, this is evidence pointing to a gonorrheal infection in the urethra.

Addition of a preservative to the urine will prevent any changes taking place in pus cells. Red cells and casts diminish in numbers after a few days. There is an increase in the inorganic sediment.

Reaction.—The reaction of urine changes on standing. Its estimation is only of value when the fresh specimen is examined. Litmus is relatively insensitive and will detect only large variations in reaction. Frequently a neutral (amphoteric) reaction is found. Papers dipped in the universal indicator are satisfactory and recently the La Motte Company have devised a simple apparatus for estimating the hydrogen-ion concentration. An ammoniacal odor and alkaline reaction are present when urinary bacteria break up the urea into ammonia.

Interpretation.—The normal reaction of urine is usually acid but may be neutral or at times alkaline. In urinary infections the reaction is alkaline in cystitis but acid in pyelonephritis. In tuberculosis of the kidney the urine is practically always acid. In metabolic disturbances the urine reaction is of considerable importance. In acidosis the urine is continually acid and can only be rendered alkaline by large doses of sodium bicarbonate. This forms a valuable clinical test and treatment for acidosis. (See page 165.)

Alkalosis occurs in obstructing gastric ulcers or severe vomiting as well as with alkali therapy. The urine is then usually alkaline due to the presence of fixed alkalies rather than urinary fermentation. This is a valuable diagnostic test for alkalosis and the state may be corrected by administration of ammonium or calcium chloride.

The food and drug intake influence the reaction of the urine. Two or three hours after a meal the acid reaction is lessened or at times an alkaline reaction appears. This is due to the temporary absorption of the acid by the foods in the stomach and the greatly increased output of urine diluting the acidity. Apart from this, proteins tend to produce an acid urine and fruits a neutral or alkaline urine. Even acid fruits produce an alkaline urine because the acid is organic and is oxidized leaving the base free to join up with acids. Sodium or potassium citrate or sodium bicarbonate will render the urine alkaline. Ammonium chloride will render it acid because the ammonium is oxidized leaving the chlorine free to combine with fixed bases.

Specific Gravity.—Specific gravity is one of the most important tests in estimating renal function. The procedure is very simple but before placing reliance in the results we should make sure that the readings are correct.

Twirl the urinometer as it goes into the urine. This will prevent it adhering to the side of the container before finding its correct level. It will also remove any bubbles which might adhere to the bulb, buoying it up so as to give an unduly high reading.

The urinometer should be a large one with the stem 3 inches long and graduated from 1.000 to 1.040. Graduation to 1.060 crowds the spaces too much and from 1.040 to 1.060 is rarely of use. The very small urinometers with close graduations are worse than useless. Each urinometer should be tested and this is very easily done by immersing it in tap-water at room temperature when the reading should be exactly 1.000. It is well to test them also at a higher point. An accurate 5 per cent solution of sodium chloride, chemically pure, will give a specific gravity of 1.035 at 25° C. (or 77° F.)

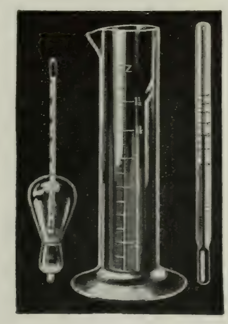


FIG. 75.—Tycos urinometer (high-grade) with thermometer for temperature correction.

The specific gravity of a urine at body temperature is 5 points lower than at room temperature. For urines at extreme temperatures, corrections should be made by adding 0.001 to the urinometer reading for each 3° C. above the temperature for which the urinometer is standardized or subtracting 0.001 for each 3° C. below. The Tycos urinometer is supplied with a thermometer specially graduated for making quick corrections. If there is insufficient urine submitted to float the urinometer it is better to obtain another sample than to dilute the urine with an equal quantity of tap-water and multiply the result by 2.

Interpretation.—The specific gravity depends upon the amount of dissolved waste products in the urine. Nine-tenths of the waste products of the body are excreted by the kidney. The normal kidney is capable of excreting urine with specific gravity varying from 1.003 to over 1.030. If the fluid intake be large the output is large and the specific gravity is low except in diabetes. If the fluid intake be small it will be high. Loss of fluid by perspiration or diarrhea also tends to increase the specific gravity.

Ordinary night urine should have a specific gravity of over 1.020 and be under 400 cc. in amount. The best diagnostic information on renal function is obtained from the urine-concentration test. A specific gravity around 1.030 and not less than 1.025 signifies good concentration of the waste products by the kidney. Much the same information is obtained from the two-hour test of quantity and specific gravity.

The total solids in the urine is another means of investigating the kidney output of waste. The urea, which is slightly more than one-half the total solids in urine, has approximately the same percentage in a specimen of urine as the last two figures of its specific gravity. Approximately 1 per cent of sodium chloride occurs in normal urine and accounts for 1.007 of specific gravity. It forms about one-half the inorganic salt content of the urine. The last two figures of the specific gravity multiplied by 2.6 gives approximately the grams of total solids per liter of urine. The estimation should be made on the twenty-four-hour specimen and when the amount is over a liter and the specific gravity 1.019 or over to make the total solids over 50 gm. there is adequate output of waste material from the body. Deductions regarding the urea and salt content only hold good when the patient is on normal diet. Excessive ingestion of salt will alter the relationships.

A low fixed specific gravity indicates a chronic interstitial nephritis with considerable damage to the kidney glomeruli and probably urea retention. A high specific gravity and albumin in a diminished total urinary output occurs in parenchymatous nephritis where the renal tubules are degenerated resulting in chloride retention and edema.

MICROSCOPIC URINE ANALYSIS.

Amounts of pus or blood may be estimated by the height of the sediment in the centrifuge tube provided other substances are not also present in the sediment. Mucus frequently produces an indefinite upper level in the sediment and crystals may be present, increasing the amount.

After centrifuging four minutes pour off all the top urine. Mix the drop of urine that remains with the sediment by striking the tube against the hand, then pour out a small drop on a glass slide. Place a $\frac{7}{8}$ inch cover glass on the drop of sediment. Only one layer of cells should be seen. If the drop is too large many layers will be present and obscure the examination. To remedy this press down the cover-glass, blotting away the excess fluid that appears at its margins.

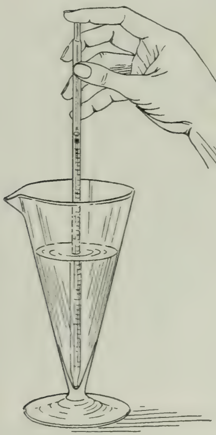


Fig. 76.—Obtaining a urinary sediment, after it has had an hour to settle.

As a substitute for centrifuging, sedimentation by gravity in a conical glass or tall test-tube for at least one hour will enable all the pus, much blood and many casts if present to settle. With the finger-tip over a pipet insert its tip down to the sediment. Tilt the finger over the end of the pipet slightly so that a small amount of the sediment will come up in the tip. Transfer this drop of sediment to a glass slide, add a cover-glass and examine.

The important findings are pus, blood and casts. When pus is degenerated or the urine alkaline it is difficult to distinguish pus cells from blood cells. The following points are important:

1. Pus cells are almost twice as large as red blood cells.
2. Pus cells have granular centers, red cells have a shiny surface with a bluish-green tint when fresh and may have a crenated outline. They lose this color on standing in urine.

If there is still doubt the following may be resorted to:

1. Transfer a small drop of blood from a finger puncture to the edge of a coverslip for comparison or

2. A drop of acetic acid placed on the slide renders all red cells invisible. Do not forget the few leucocytes present in the blood or

3. Dry the sediment, fix and stain it with methylene blue and examine under the oil-immersion lens. The irregular nuclei of the pus cells stain a deep blue while the red cells stain a yellowish-green.

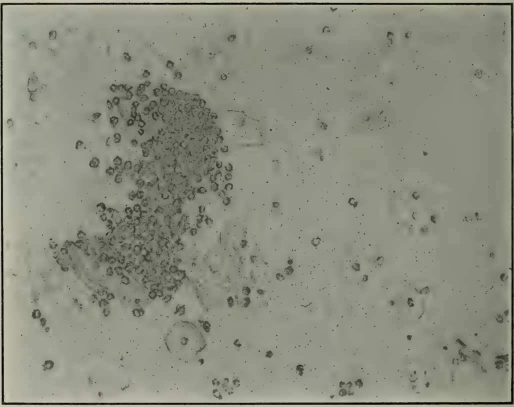


FIG. 77.—Urinary sediment × 100. Clumped pus, suggestive of chronic gonorrheal infection.

Do not confuse casts with cylindroids, mucus threads or fibers of cotton. A cast should have two parallel sides, be at least three times as long as it is broad and have two rounded ends. Many casts are broken off but at least three or four perfect specimens should be seen before reporting casts present. *Neither end should taper and come to a tail as do cylindroids.* Scratches on glass have many striations otherwise they may resemble casts. In searching for casts use a very subdued light by partially closing the diaphragm. Their outline is best seen by moving the focus of the microscope while searching for them. In this way the hyaline casts will not be missed. It is important to note the degree of granularity of casts. In pyelonephritis, casts are frequently covered with pus cells and in acute disease with red blood cells. Usually several varieties are seen but there will be one main type. It is important to distinguish the very broad granular and epithelial casts which form in the larger collecting tubules. These casts are of serious import. Casts are usually found where there is albumin of renal origin. Most casts

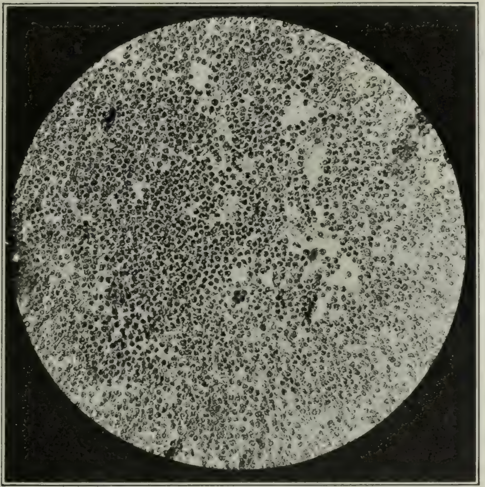


FIG. 78.—Urinary sediment × 100; much pus.

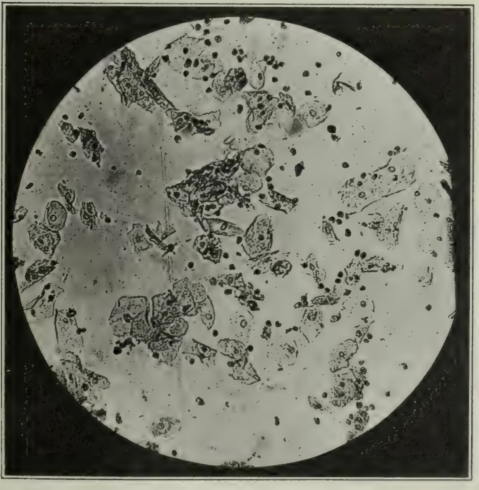


FIG. 79.—Urinary sediment × 100 under low-power lens and cover-glass. Squamous epithelial and a few pus cells. This has no urological significance. It is vaginal epithelium with pus from a mild endocervicitis.

have a homogeneous or granular matrix to which may adhere pus, blood or epithelial cells. The essential points in detecting casts are fresh urine, a well closed diaphragm to obtain subdued illumination and the use of the low-power lens.

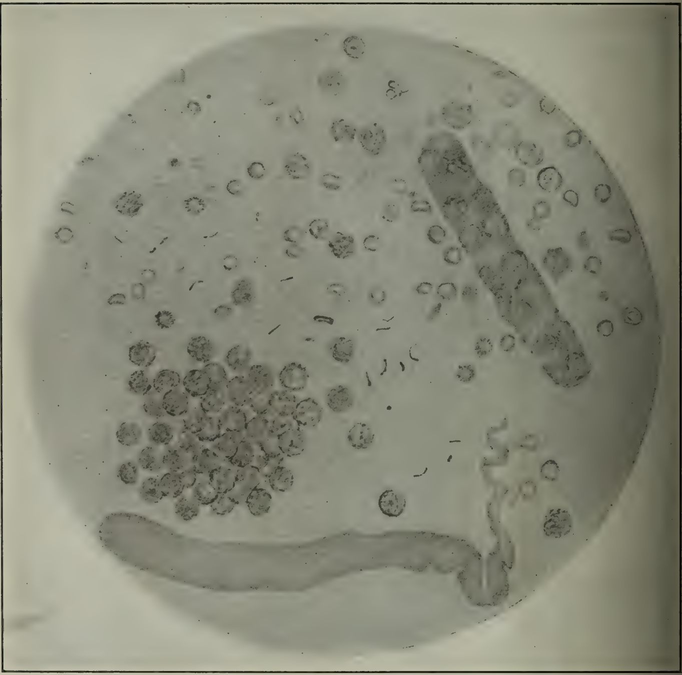
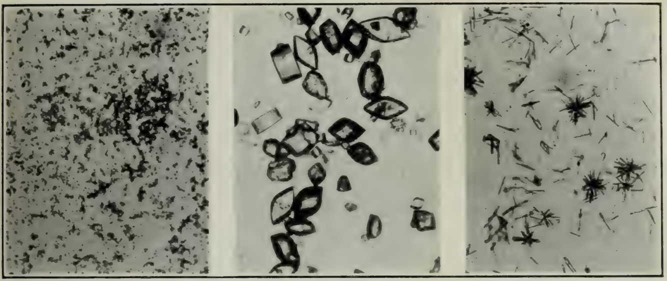


FIG. 80.—Urinary sediment × 450 (high dry lens and × 10 eyepiece). The granular pus cells are almost twice the size of the smooth red cells which when fresh have a greenish tint. Other bodies from above down are: a granular pus cast, bacteria, a clump of pus and a cylindroid.

Cylindroids and mucus when small in amount are normal and of no diagnostic significance. The cylindroids come from the renal tubules and the mucus strands are discharged from mucous glands at the juncture of the urethra and bladder.

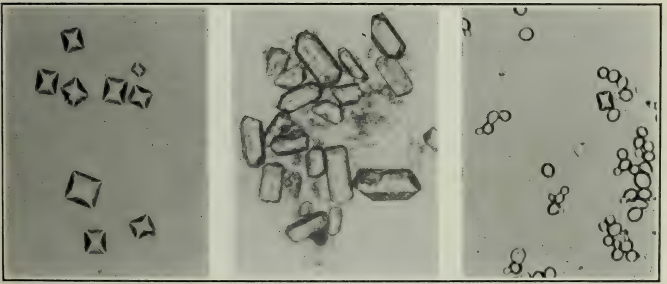
Spermatozoa are seen under the high dry lens as oval bodies with long delicate tails. When seen occasionally they are of no impor-



Amorphous deposit (urate in acid, phosphate in alkaline urine).

Uric acid in acid urine.

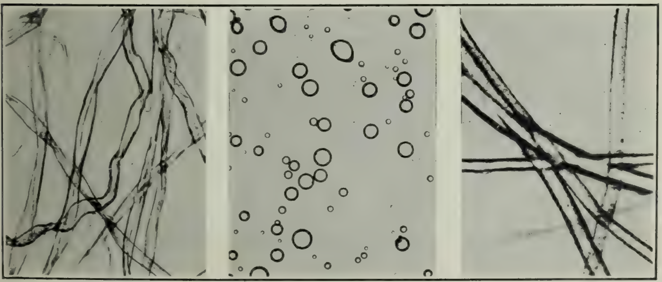
Calcium phosphate (stellar phosphate) in feebly acid, alkaline or neutral urine.



Calcium oxalate in neutral, acid or alkaline urine.

Ammonium magnesium phosphate (triple phosphate in alkaline urine only).

Yeast (× 450) resembling red blood cells but some are oval and show lateral buds.



Fibers of absorbent cotton.

Fat droplets; toluol. They resemble red blood cells but vary in size.

Fibers of wool.

Fig. 81.—Objects of no pathological significance in urine sediment. × 100 (low-power lens, × 10 ocular.)

tance. They may be seen in the urine of males or females after coitus.

When epithelium, mucus and a small amount of pus are present in the urinary sediment the specimen is usually from a female and the cells are from the cervix uteri rather than from the urinary system. Apart from this, epithelial cells have no diagnostic significance.

Crystals and various precipitates are of little diagnostic importance. They occur in urines of high specific gravity when they have stood for a time, especially during the cold weather. Because they are frequently seen, they are briefly described. Amorphous sediment usually consists of (1) urates, which occur only in acid urines and dissolve on heating, (2) phosphates, usually white in color occurring in alkaline urines. The precipitate is augmented by heat but dissolves on adding acid. (3) Carbonates which dissolve on adding acid with the evolution of carbon dioxide gas.

Crystals.—(*a*) *In Acid Urine.*—*Uric acid* which consists of yellowish-brown squares or wedges.

Oxalic acid, squares with 2 diagonal lines which intersect. These are frequently found in neutral or slightly alkaline urines. Occasionally large dumb-bell forms are seen.

(*b*) In *alkaline urine. Ammonium magnesium phosphates* (triple phosphate)—a prism within a prism, sometimes takes feathery form.

Calcium hydrogen phosphates (stellar phosphates).—Long crystals converging at a point.

Calcium carbonate—small dumb-bell forms.

Ammonium biurate crystals—yellowish or brownish spheres sometimes showing spicules.

There are many other rare forms of crystals.

Quantitative Microscopic Urine Examination.—To overcome the inaccuracy caused by sedimentation, transferring sediment to slide, varying thicknesses of films examined, Exton has outlined a quantitative microscopic examination for urinary findings.[1]

Equipment.—1. Special slide with a trough $\frac{1}{10}$ mm. deep and 20 mm. square. Manufactured by Bausch and Lomb Optical Company.

2. Pipet with rubber bulb suction for filling and emptying centrifuge tubes.

3. Centrifuge tubes scratched at 0.5-cc. and 15-cc. marks.

Technique.—1. Fill an ordinary centrifuge tube up to the 15-cc. mark with well mixed urine.

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[1] Exton: J. Lab. and Clin. Med., 1930, **15**, 386

2. Centrifuge four and one-half minutes at 2000 revolutions per minute.

3. With the pipet withdraw the 14.5 cc. of supernatant urine and, using the end of the pipet mix the remaining 0.5 cc. of sediment plus urine.

4. Transfer this emulsion of sediment to the trough and cover with the thick cover-glass.

5. Examine under the microscope using × 10 eyepiece with 16 mm. (low power) or 4 mm. (high dry) objective with diminished light.

6. Count the cells in four fields under the low power in well mixed specimens. Multiply the average of the cells per low-power field by 170 to obtain the number of cells per cubic centimeter of urine. If the high power be used in densely cellular specimens count 10 fields and multiply the average of the cells per high-power field by 3,100 to obtain the cells in a cubic centimeter of urine.

Interpretation in Detail of the Microscopic Examination.—A microscopic examination should be done as a routine because a urine containing small and at times moderate amounts of pus which shows as 100 pus cells per high-power microscopic field may not give a positive albumin reaction. Less than 20 red blood cells per high-power field and a few casts in the sediment may not be accompanied by albumin. These microscopic findings are frequently the most valuable feature of the urine examination.

It is probably best to interpret the microscopic findings as follows:

Pus.	
Few pus cells (note if in clumps)	**10 to 25 per high-power field.**
(Less than 10 are disregarded.)	
Frequent pus cells	**25 to 100 per high-power field.**
Much pus	**100 or over per high-power field.**
Red Blood Cells:	
Few red cells	**4 to 10 per high-power field.**
(Less than 4 are disregarded.)	
Frequent red cells	**10 to 25 per high-power field.**
Many red cells	**25 or over per high-power field.**
(The average of 10 high-power fields should be taken.)	
Casts:	
Few casts	**3 to 10 in the whole sediment.**
Frequent casts	**10 to 25 in the whole sediment.**
Many casts	**over 25 in the whole sediment.**

Interpretation of Pus.—Pus in the urine may occur from an infection anywhere along the urinary tract from the kidney substance to the end of the urethra and the amount of pus depends on the severity and extent of the infection. Always employ the two-bottle test as outlined on page 207 to determine the location of the infection. In males exclude prostatitis by examination of the prostatic secretion and in females exclude vaginal discharge due to a cervicitis by having the patient sponge off the genitalia or take a douche before urinating.

When pus is present always examine the urine for bacteria if it be fresh. A culture is not usually necessary. Centrifuge fresh urine for ten to fifteen minutes and examine stained smears of the sediment for bacteria. It is necessary to have fresh urine so that no change may occur in the bacterial picture. It is surprising how frequently this will reveal streptococci and staphylococci as well as Bacillus coli. Pus with no pyogenic bacteria should make one suspect tuberculosis. A cystoscopic examination is valuable here. Pus from a pyelonephritic is frequently intermittent, especially if associated with abscesses of the kidney. Pus resulting from a renal calculus or tuberculosis is usually constant. In cystitis the amount of pus is constant but in urethritis there is more pus in the first bottle specimen than in the second.

Pyelonephritis in Children. Obtaining the Specimen.—If in the home give the child a maximum of fluids, hold it on the knee and when urine comes away catch same in a bowl.

In hospital a specimen may be best collected from an infant by strapping between the thighs with adhesive, so as to cover the urethra, a small beaker or large test-tube. Failing this a urine may be collected by placing the infant on a rubber sheet. No cloth should come in contact with the specimen as it may take up the pus cells.

A method of examination commonly employed is to place a drop of the shaken-up urine, uncentrifuged, on a glass slide, add a cover-glass and examine with the low-power lens. Take the average of the number of cells counted in half a dozen microscopic fields. Over 3 are abnormal in males and over 8 in females. The most satisfactory method if the Exton equipment (page 294) is not at hand, is to place a drop of the shaken-up urine in the counting chamber and count the cells in the ruled area which in the Neubauer ruling consists of 9 squares each a millimeter deep. The high-power lens is necessary to distinguish leucocytes from the small round epithelial cells. Count 2 drops and take the average. Ten-ninths of the average is the number of cells per cubic millimeter of urine. This count is the most accurate method of estimation and takes only five minutes. The progress of the case may be followed by cell counts in the urine although they are apt to vary in an irregular fashion at times.

Interpretation of Urinary Leucocyte in Children.—The normal is around 10. It is usually less than 20 cells per cu.mm. although 50 may be taken as a top limit. The higher counts occur more frequently in girls. Counts of from 50 to 100 occur in general non-urinary infections. Counts of over 100 indicate an infection in the urinary tract if vaginitis be excluded. In quiescent or chronic pyelonephritis there are usually less than 500 cells. A slight turbidity is seen when 400 cells are present. In acute pyelonephritis from 500 to 5000 pus cells are present per cu.mm. (1000 cu.mm. = 1 cc.)

In children when there is a high fever of unknown origin, always suspect pus in the urine. Finding it will frequently explain the cause of a persistent restlessness, irritability, fever, rigor or chronic wasting.

Blood.—Blood in the urine is always serious and nearly always means local disease in the kidney, bladder or prostatic urethra. Among the common causes are:

1. Nephritis or passive congestion of the kidney due to heart failure.
2. Calculi in the kidney or ureter.
3. Malignant papilloma of the bladder or renal carcinoma which may be accompanied by calculi, adenomatous hypertrophy or carcinoma of the prostate.
4. Tuberculosis of the kidney.
5. Chronic infection, pyelonephritis, cystitis, prostatitis.

In nephritis casts will usually be found with the red blood cells. Rare causes are purpura,[1] and other blood diseases, foreign body and malformations. Essential hematuria should be allowed as a diagnosis only when all causes have been excluded by a complete examination. A two-bottle test will help to locate the site of bleeding. When the source of the hemorrhage is in the bladder the largest amount of blood usually appears just at the last of the second specimen. Pain usually precedes or accompanies the bleeding due to renal or ureteral calculus. Cystoscopic examination is valuable in locating the cause and it should be performed while the patient is bleeding. Twenty-five per cent of all urological patients have hematuria as a sign. The hematuria is macroscopic in about 50 per cent of cases and microscopic in the other 50 per cent.

Casts.—Casts are usually found where there is albumin of renal origin. They are an expression of injury on the part of the cell lining the renal tubules. Casts may be present in the urine when only a small portion of one kidney is inflamed. Hence one cannot always form an opinion regarding the severity of a nephritis from examination of the urinary sediment.

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[1] Locke and Minot: Jour. Am. Med. Assn., 1924, **83**, 1311.

Certain points are of value however. Hyaline casts occur temporarily in health especially after a severe physical exertion. Their constant presence signifies kidney damage. The process is more severe when granular and epithelial casts are present. Large numbers of coarsely granular casts indicate a more diffuse inflammatory process which is of serious import. Blood casts occur in acute disease and pus casts with infective pyelonephritis. The very broad, coarsely granular type are called renal failure casts and when present in large numbers indicate a marked degree of renal failure with elevation in the blood urea over 100 mgm. per 100 cc.[1] They come from the larger collecting urinary tubules.

NEPHRITIS.

Volhard and Fahr classification.	Corresponding classifications.	Laboratory findings.
A. Nephrosis (degenerative tubular)	Chronic parenchymatous, hydremic or wet nephritis; chronic nephritis with edema	Normal blood-pressure; normal blood urea; high specific gravity; large amount of albumin; numerous granular casts.
B. Inflammatory nephritis:		
I. Diffuse glomerulonephritis:		
1. Acute stage, intracapillary or capsular	Acute nephritis	High blood pressure; elevated blood urea; albumin, in acute stage and terminal.
2. Chronic stage, no evidence of renal insufficiency.		
3. Terminal stage with renal insufficiency	Dry; chronic interstitial or azotemic nephritis	Large amounts of urine; low specific gravity; polyuria; nocturia; few casts.
There may be added a degenerative tubular nephrosis in acute and terminal.	Wet nephritis, nephritis with edema; parenchymatous or hydremic.	As above in nephrosis.
II. Focal:		
1. Acute:		
(*a*) Interstitial non-suppurative . . .	*Nil*	No change in findings.
(*b*) Embolic suppurative.	.....	May find few red cells in embolic nephritis.
2. Chronic.		
C. Arteriosclerotic kidneys:		High blood-pressure
1. Simple sclerosis of renal arterioles.	.....	
2. Extension of endarteritis to produce sclerosis of glomeruli	Arteriosclerotic kidney; dry or chronic instertitial nephritis	Low specific gravity of urine; polyuria; few casts; increased blood urea.

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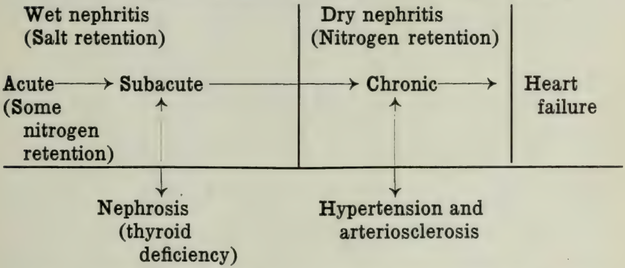
[1] Addis: Jour. Am. Med. Assn., 1925, **84**, 1013.

Bacteria.—When pus and bacteria are present, smears of the sediment are of value in determining the type of organism present. Only fresh specimens are suitable for examination of bacteria and they must be obtained with aseptic precautions before a culture is of value. (See page 319.)

CLINICAL CLASSIFICATION OF NEPHRITIS. (GILMOUR.)


Wet nephritis
(Salt retention)
Dry nephritis
(Nitrogen retention)
Acute
(Some nitrogen retention)
Subacute
Chronic
Heart failure
Nephrosis (thyroid deficiency)
Hypertension and arteriosclerosis

Acute nephritis is a diffuse inflammation involving the glomeruli and tubules of the kidney. Throat infections very often precede or accompany it. The onset is sudden and the patients frequently complain of headache and backache, nausea and perhaps vomiting, diarrhea and dyspnea. There is usually edema of the legs, face and sometimes of the body. During the early stages the blood urea and other non-protein substances are increased.

TYPICAL URINARY FINDINGS IN A MODERATELY SEVERE CASE OF ACUTE NEPHRITIS. PHYSICAL AND CHEMICAL EXAMINATION.

Color—dark yellow.
Specific Gravity—1.020.
Albumin—large amount (1 per cent).
Transparency—smoky, opaque.
Reaction—acid.
Sugar—negative.

Microscopic Examination.

Casts
- Hyaline—none.
- *Granular—numerous.*
- *Blood—numerous.*

Red Blood Cells—100 (per high-power field).
Pus cells—20 (per high-power field).

Examination of a Twenty-four-hour Specimen.

			Normal.		Normal.
Amount	Day—	240 cc.	750–1200 cc.	Chloride, 1.26 gm.	10–15 gm.
	Night—	180 cc.	150– 300 cc.		
	Total—	420 cc.	900–1500 cc.	Urea, 7.56 gm.	25–35 gm.

30 cc. = 1 ounce.

The patient may recover entirely or go on to the subacute stage.

Subacute Nephritis.—Synonyms: Chronic nephritis with edema, parenchymatous or hydremic nephritis.

It may follow acute nephritis or develop gradually without the acute attack. A throat infection usually precedes it however. Lack of energy and weakness are common complaints and edema is the most constant sign. The blood-pressure is moderately increased (150 to 200 mm. of Hg.) and there is a tendency to elevation of the blood urea nitrogen at the later stages.

TYPICAL URINARY FINDINGS IN A MODERATELY SEVERE CASE OF SUBACUTE NEPHRITIS.

Color—light yellow.
Specific Gravity—1.020 *to* 30
Albumin—large amount 1 per cent.

Transparency—slightly turbid.
Reaction—acid.
Sugar—negative.

Microscopic Examination.

Casts
- *Hyaline—numerous.*
- *Granular—numerous.*
- *Epithelial—numerous.*

Red Blood Cells—20 (*per high-power field.*)
Pus Cells—10 (*per high-power field.*)

Examination of Twenty-four-hour Specimen.

		Normal.		Normal.
Amount	Day— 360 cc.	750–1200 cc.		
	Night—240 cc.	150– 300 cc.	*Chloride*, 2.46 *gm.*	10–15 gm.
	Total—600 cc.	900–1500 cc.	Urea, 24 gm.	25–35 gm.

The prognosis is unfavorable, the end coming in a year or two or the disease passing over into the chronic dry nephritis with nitrogen retention.

Chronic Nephritis.—Synonyms: Small granular kidney, chronic interstitial or azotemic nephritis.

This stage may follow the wet subacute nephritis or it may be the terminal picture of cardiovascular hypertension. Diminished energy, early exhaustion and nocturia are leading symptoms and hypertension with cardiac enlargement the commonest signs.

The lesion mainly involves the glomeruli of the kidney and there is retention of urea and other non-protein substances. In hypertension the renal arteries show thickness which later extends to involve the glomerulus so that the end result is the same as in the type following subacute nephritis.

Typical Urinary Findings in a Moderately Severe Case of Chronic Nephritis.

Physical and Chemical Examination.

Color—watery.
Specific Gravity—1.009.
Albumin—small amount (0.01 per cent).
Transparency—clear.
Reaction—acid.
Sugar—negative.

Microscopic Examination.

Casts
- *Hyaline—few.*
- Granular—none.
- Blood—none.

Red Blood Cells—0 to 10 (per high-power field).
Pus Cells—none (per high-power field).

Examination of a Twenty-four-hour Specimen.

		Normal.		Normal.
Amount	*Day*— 1100 cc.	750–1200 cc.		
	Night— 950 cc.	150– 300 cc.	Chloride, 12 gm.	10–15 gm.
	Total— 2050 cc.	900–1500 cc.	Urea, 14.35 gm.	25–35 gm.

The disease is progressive and when heart failure occurs general edema and renal stasis are added.

Nephrosis.—Nephrosis is a degenerative lesion involving the tubules of the kidney. It commonly occurs during the high fever of an infectious disease. This type usually clears up shortly after the fever ceases. It may begin insidiously or as the result of a chronic toxemia. The patient has surprisingly little disability in view of the edema and marked albuminuria. There is a general metabolic disturbance in which the basal rate is subnormal. Normally there is twice as much albumin as globulin in the blood. In nephrosis the albumin is only one-half the globulin. After treatment with high-protein diet and thyroid extract, elimination of the waste in the body fluids causes a temporary increase in the blood urea nitrogen. In the untreated the urea is normal. The blood-pressure is never increased.

Typical Urinary Findings in a Moderately Severe Case of Nephrosis.

Physical and Chemical Examination.

Color—light yellow.
Specific Gravity—1.025 to 1.040
Albumin—large amount (1 *per cent*).
Transparency—slightly turbid.
Reaction—acid.
Sugar—negative.

Microscopic Examination.

Casts	*Hyaline—numerous.*	Red Blood Cells—none (per high-power field).
	Granular—numerous.	Pus Cells—none (per high-power field).
	Blood—none.	

Examination of a Twenty-four-hour Specimen.

		Normal.		Normal.
Amount	*Day*— 420 cc.	750–1200 cc.		
	Night—275 cc.	150– 300 cc.	*Chloride,* 3.5 *gm.*	10–15 gm.
	Total— 695 cc.	900–1500 cc.	Urea, 21 gm.	25–35 gm.

The disorder may continue for years or it may clear up. The end result in many cases involves the glomeruli changing the picture to one of chronic nephritis.

URINE TESTS FOR OCCASIONAL USE.

COLLECTION OF a TWENTY-FOUR-HOUR SPECIMEN.—No quantitative test is of value unless performed on a sample of a mixed twenty-four-hour specimen. The physician should inform himself as to the patient's fluid and food intake. The following definite instructions should be given to the patient. Empty the bladder at 8 A.M. and discard the specimen. Collect in a clean 80-ounce bottle all urine passed until 8 o'clock the following morning and at that time empty the bladder, adding it to the collected specimen. The patient should collect the urine before moving the bowels; otherwise considerable urine will be lost. After mixing the specimen well, measure the whole quantity in ounces, record the amount and bring a sample of the mixed urine in a 4- to 8-ounce bottle for examination.

Quantity Passed in Twenty-four Hours by an Average Sized Healthy Adult. (Weight 70 kilos or 150 pounds).—The output of urine is roughly equal to the liquid intake of the diet, the water in vegetables and other foods supplying the moisture lost by respiration and perspiration. The optimum urine output is 1000 to 1500 cc. (35 to 50 ounces). If the water intake is low or perspiration marked the urine output may in normal persons, be as low as 500 cc. (17 ounces). With a large water intake or in cold weather the urine output may be up to 2500 cc. (83 ounces). The normal output during the day (8 A.M. to 8 P.M.) is two or three times the output during the night (8 P.M. to 8 A.M.). If they become equal it is a sign of an overloaded kidney working overtime. Undue frequency of urination is an important symptom usually denoting cystitis or urethritis. Occasionally overflow incontinence may be a cause especially in elderly men.

Water Tests for Renal Function.—The first and most reliable test to employ is the simple urine concentration test of Addis.

PRINCIPLE.—Kidney function consists in the concentration and removal from the blood of waste products which are then excreted in the urine. There is no serious impairment of renal function if the specific gravity of the urine can be elevated to 1.025 or over.

By the following technique restriction of fluids is obtained for a period of twelve to fifteen hours mostly while the patient sleeps. This is a convenient arrangement and avoids distress from thirst.

Instructions to the Patient.—About 6 P.M. eat a usual supper barring fluids of any kind such as water, tea, coffee, milk or soup and do not eat or drink again until the test is completed. Empty the bladder at bedtime, discarding the specimen. On waking in the morning empty the bladder and save the urine in a bottle marked No. 1. Remain in bed and an hour later collect another specimen in a separate container labelling it No. 2. If the hour is early, a third specimen may be collected two hours after waking.

The Test and Interpretation.—Take the specific gravity of specimens saved, observing precautions for accuracy laid down on page 287.

If the patient's renal function is competent the maximum specific gravity will be over 1.030, frequently 1.032 to 1.034. The lowest figure that can be considered a normal maximum is 1.025. In assaying results a high reading is more reliable than a low reading. Low readings may be due to (1) the patient failing to follow instructions; (2) eating a diet which has a very low-salt and low-protein content or (3) the excretion of retained fluid from edematous tissue.

These ruled out, a maximum figure below 1.025 indicates an impaired renal function and if the maximum is only 1.020 there is a very serious kidney defect.

At the same time the blood urea and other non-protein constituents may be normal because of a diminished protein intake or because of a compensating polyuria. A twenty-four-hour specimen with a specific gravity of 1.011 and a volume of 2500 cc. contains the same amount of waste product as if it had a specific gravity of 1.022 and a volume of 1250 cc. The water excretion is also less than in a normal kidney and only by continuous action does it excrete large amounts.

Finally this polyuria gradually fails, producing a retention of nitrogenous waste in the blood as demonstrated by the increased readings of the blood urea nitrogen. The water-dilution test now shows a greatly delayed excretion. After drinking 1200 cc. less than 400 cc. are excreted during the first four hours.

The concentration test is the first to indicate deficient renal function in the advancing arteriosclerotic kidney or in early glomerulonephritis. Here there may be no albumin even after nitrogenous retention develops, but the patient usually has nocturia.

When heart failure develops the specific gravity of the urine is at a continuous level of about 1.020 but the volume is seriously diminished.

The Specific Gravity Test of Volhard.—This test is more inconvenient to the patient than the urine concentration test. Its result is influenced by circulatory as well as renal inefficiency and it shows more positive findings in nephrosis than does the water-concentration test.

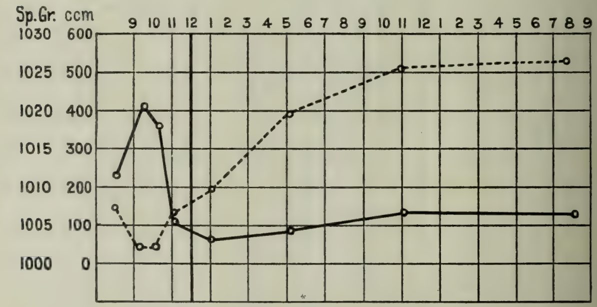


FIG. 82.—Water test. Normal result. A liter of water is taken on an empty stomach at 8 A.M. Eleven hundred cubic centimeters are excreted within four hours and the specific gravity drops to 1.002. Later in the day on a dry diet the specific gravity mounts to over 1.025 and the amount of urine is small.

The Test.—In the early morning the patient empties his bladder and then drinks 1200 cc. of water over a period of one-half hour. During the day nothing but a dry diet is eaten. Measure the amount and save a sample of all specimens passed during the day.

Result.—A normal person will secrete 1000 to 1200 cc. during the first four hours following the ingestion of the water. Some of the specimens will have a specific gravity as low as 1.003. Late in the day the amount of urine decreases and by the late afternoon the specimens will have a specific gravity as high as 1.025.

In passive congestion of the kidney due to heart failure, in nephritis showing edema, or in advanced chronic nephritis without edema the specific gravity may not go lower than 1.005 or 1.010 and there may be as little as 400 cc. excreted in the four hours following the drinking of the water. There will be a delayed excretion of the water as long as twelve hours after.

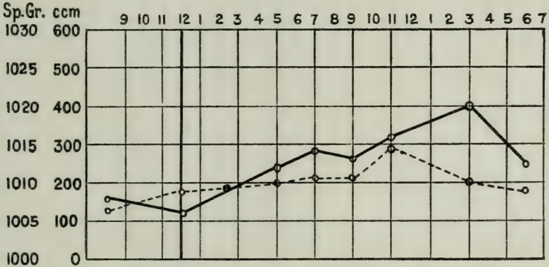


FIG. 83.—Water test in chronic nephritis with a mild degree of heart failure. A liter of water is taken on an empty stomach at 8 A.M. There was no immediate increase in the output of urine. The patient felt abdominal discomfort. The specific gravity remained 1.007 to 1.014. The output of urine increased after the patient reclined at 6 P.M.

There are many modifications of this test, some requiring a standard diet, set hours for passing the urine, one day for diuresis and the next day for the concentration test but the information obtained from all is much the same.

The Two-hourly Quantity and Specific Gravity Test.—The two-hourly quantity and specific gravity test as outlined by Schlayer and Hedinger and modified by Mosenthal is popular with some clinicians. Its technique is as follows but it seems almost impossible to collect all the specimens at the proper hours.

Instructions to Patient.—Eat a usual meal at 8, 12 and 5 o'clock with about 600 cc. (20 ounces or 1 pint) of fluid at each meal. Do not eat or drink between meals. Empty the bladder at 8 A.M and discard the specimen. Save in separate containers specimens voided at 10 A.M., 12 M., 2, 4, 6, 8, 10 P.M., the night urine passed from 10 P.M. up to and including the specimen passed at 8 A.M. next morning and label accordingly. Measure the quantity of each specimen and estimate its specific gravity with the urinometer and record them opposite the time at which the specimen was passed. The important points in a normal case are:

1. A night urine of under 400 cc. in quantity.

2. The total amount excreted over 1200 cc.

3. Variations in specific gravity of the day urine of 10 points or more and a night urine with a specific gravity of 1.016 or over.

Pathologic Findings.—1. When kidney function becomes impaired it is first manifested by an increase in night urine which indicates an overworked kidney. Over 400 cc. is suspicious and over 700 definitely abnormal.

2. The total amount will be under 1200 cc. in tubular (chronic parenchymatous) nephritis and in heart failure. The specific gravity will be fixed at a high level.

3. A low fixed specific gravity where the highest is not over 1.016 and there is less than 10 points variation is a serious sign of nitrogenous retention. There is a compensating polyuria. If it fails death will occur shortly.

The low-fixed specific gravity is found most frequently in advanced glomerular (chronic interstitial) nephritis but it may be found in anemia, polycystic kidney, pyelonephritis, hypertrophied prostate and urethral stricture.

Phenolsulphonephthalein Test.—*Apparatus and Reagents.*—Ampules of sterile dye containing slightly over 1 cc. of fluid with 6 mgm. of the dye per cubic centimeter obtained from Hynson, Westcott & Dunning.

Sodium bicarbonate.

One liter measure.

One tuberculin syringe, 1 cc. with 23- or 24-gauge needle.

Alcohol and absorbent or gauze sponges.

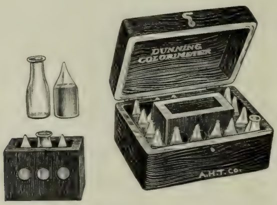


FIG. 84.—Dunning colorimeter for estimating the percentage of phenolsulphonephthalein excreted as an index of renal function.

The Dunning colorimeter is convenient. A test-tube colorimeter with freshly compared standard is satisfactory and the margin of error is less than 7 per cent.[1]

Obtaining the Specimen.—Give the patient two glasses of water to promote secretion of urine. After five to ten minutes have him empty his bladder. Discard the urine and inject exactly 1 cc. of the

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[1] Preparation of the standard: Add 1 cc. of the phenol dye from an ampule to about 800 cc. of water. Add 1 dram of the sodium bicarbonate to bring out the color of the dye and dilute to 1000 cc. Since this contains the same amount of dye as is injected it may be rated at 100 per cent standard color solution. It keeps in a well-stoppered bottle for three months.

"phthalein" from the ampule into a prominent vein at the elbow according to the technique outlined on page 197. Collect specimens of urine one-half hour and one hour after injection.

The Test.—Measure the first specimen of urine, add about a dram of powdered sodium bicarbonate. Dilute to 1000 cc. and filter a sample for colorimetric estimation. With the Dunning colorimeter the sample to be tested is placed in the open ampule and compared with the standard. If this is not at hand the percentage may be estimated by comparing the standard solution with the unknown in test-tubes as outlined on page 394.

Intravenous Compared with Intramuscular Injection and the Normal Output.—The intravenous route is chosen for injection to avoid the absorption hazard when the dye is injected intramuscularly or subcutaneously into tissues that have sluggish circulation, edema or fat. These would delay absorption so that little or none of the dye would come through the kidneys even when they are normal. The intravenous injection of the dye is not at all dangerous. For hurried office practice, it is best to take a specimen of urine fifteen minutes after the dye is injected. If this specimen contains over 30 per cent of the dye, the kidney function is normal and one need not proceed further with the test. The normal kidneys excrete on an average 40 per cent during the first fifteen-minute period, 17 per cent during the second, 8 per cent during the third and 4 per cent during the fourth.[1]

If the half-hour period is taken, over 45 per cent should be normally excreted during the first half hour and 10 to 15 per cent during the second half hour. If the original intramuscular injection of the dye be employed over 50 per cent of the dye should be excreted in two hours. In health the most will be excreted during the first hour.

Interpretation.—In renal disease there is a delayed and a diminished output of the dye. The exception to this is in acute disease. In the critical acute glomerulonephritis of scarlet fever the percentage excreted may be normal. The test is valuable only in chronic disease and a normal result is good proof that the kidney function is efficient. The dye shows renal impairment nearly as early as does the water tests and long before the blood urea begins to increase.

Low readings do not always mean that nephritis is the primary trouble. In cystitis and pyelonephritis the percentage of dye excreted is unduly low and a bad prognosis should be given from the dye percentage in such states. In heart failure even when the dye is injected intravenously the dye output may be less than half normal while the blood urea is normal.

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[1] Shaw, E. C.: Jour. Urol., 1925, **13**, 575.

Prostatic and other urinary obstructions give low-excretion percentages so that it is necessary to ascertain the cause of the diminished reading.

When all causes other than renal disease can be excluded a total excretion of less than 10 per cent indicates severe renal damage and the duration of life is not likely to exceed several months.

Testing the Function of Each Kidney Prior to Nephrectomy.—After giving two glasses of water and inserting catheters in the ureters, 1 cc. of the dye is injected intravenously.

Normally, dye should come through the kidneys in two to five minutes although reflex irritation may sometimes cause a delay to eight minutes. The dye is best demonstrated by having each catheter drop into a test-tube containing a few drops of alkali. In fifteen minutes each kidney should excrete 15 per cent of the dye. If one kidney is diseased and puts out less than normal the other may compensate and put out over 15 per cent.

Immediately after removing the catheters from the ureters, the bladder should be emptied to see if any urine has leaked past the catheters while in the ureters.

A simpler and more reliable method is to estimate the specific gravity of the urine excreted from each ureteral catheter without giving the patient an excess of water.

Urea Test.

Apparatus and Reagents.—Doremus ureometer; reagents kept in two bottles.

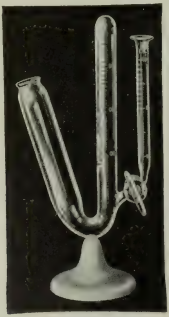

FIG. 85.—Ureometer, Doremus-Hind's model. For estimation of urea in the urine by the hypobromite method.

(*a*) Bromine—1 ounce
Potassium bromide—1 ounce
Distilled water—7½ ounces

Must be kept in a glass-stoppered bottle. Keeps one year.

(*b*) Sodium hydroxide—3 ounces
Distilled water—8 ounces

This avoids handling pure bromine which is very disagreeable.

The Test.—A mixed twenty-four-hour sample of urine should be used in every case. Equal parts of solutions (*a*) and (*b*) are mixed immediately before and used for the test. Fill the large tube and one-half the bulb with the reagent. Pour the urine into the smaller tube then turn the stopcock so as to let 1 cc. of the urine run slowly into the hypobromite solution. When bubbles have ceased to rise, read off the height of the fluid in the large tube by the graduations upon the side. The readings are in grams per liter and should be multiplied by 10 to give the percentage. Normal readings are 0.02 to 0.03 which means 2 per cent and 3 per cent.

Note.—The readings are on the whole too high for urea and approach the total nitrogen figure, but if the hypobromite solution and the urine are intimately mixed the readings will be uniform and serve satisfactorily for clinical purposes. Do a control on a normal specimen when in doubt.

Interpretation.—The urea is increased when much food is taken, also in fevers and diabetes and in poisoning by phosphorus and arsenic. It diminishes under diminished diet and severe liver disease (because less is formed) and in kidney conditions such as acute nephritis, severe glomerular nephritis and in the late stages of kidney disease where a steady decline is a very bad prognostic sign and a sudden drop a forerunner of uremia.

Urea Concentration Test. (McLean's.)

Apparatus.—Doremus ureometer; hypobromite reagent as per formula under urea estimation, ¼ pound urea.

Obtaining the Specimen.—The patient empties bladder and at once takes, on an empty stomach, 15 gm. (4 drams) of urea dissolved in 100 cc. of water. One hour after taking the urea the patient voids. Two hours after taking urea the patient again voids. The percentage of urea is calculated in three specimens as already described.

Interpretation.—According to McLean, the kidney function may be considered efficient if the urea percentage is 2 to 4 per cent. I have seen patients who have had definite renal impairment and who have been able to excrete over 2 per cent so that the test cannot be considered a sensitive one. Below 2 per cent indicates defective kidney function and the lower the concentration the more serious the lesion. Advanced cases show a concentration of only 1 per cent. Sometimes there is a marked diuresis following the administration of urea, especially if the patient has been drinking large quantities of water. If the second specimen exceeds 150 cc. the urea percentage will be unduly low.

General Remarks.—It is true that the same kidney under the same conditions may excrete at one time much and at another time little and because of this quantitative estimation, anatomical and even functional inference from the amount of any constituent excreted promises little help. The best test would be a urinary constituent which is a true end product and is capable of chemical alteration within the body and is one whose only path of excretion is through the kidneys. Urea fills these requirements to a considerable degree. Possibly the greatest objection to this test is that the rate of absorption cannot be controlled and will vary in different individuals. However, in nearly every case there will be enough absorbed after an hour to give a sufficiently high concentration in the blood and there is the advantage to be remembered that no hypodermic needle, of which the patients have a dread, need be used.

Quantitative Albumin Test. (See page 280. Sulphosalicylic Method which is the best test.)

Esbach's Approximate Method.—*Apparatus and Reagents.*—Esbach's special tube and reagent (1 gm. picric acid, 2 gm. citric acid, 100 cc. of water).

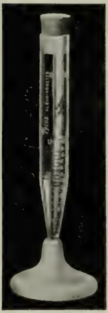

FIG. 86.—Esbach's albuminometer.

The Test.—Fill Esbach's tube with urine to the mark "U" and with Esbach's solution to mark "E." Invert the tube several times to mix the solutions. Leave overnight for the sediment to settle. The figure at the top of the precipitate indicates the number of grams of albumin per liter. Multiply by 10 to obtain the percentage.

NOTE.—The amount of albumin a nephritic patient passes at different times during the twenty-four hours varies, usually inversely to the amount of urine passed *i. e.*, with large amounts of urine the albumin percentage will be small; with diminished urine the albumin per cent will be high. The method mentioned is not an exact one so that one obtains only an approximate idea of the amount of albumin being excreted. Small amounts will register 0.01 of 1 per cent and large amount 0.15 to 0.5 per cent. The amount of albumin does not determine the seriousness of nephritis but it gives information on the albumin lost from the blood.

Quantitative Sugar Estimation.

Apparatus and Reagents.[1]

PRINCIPLE.—When urine containing sugar is heated with an alkaline copper sulphate solution containing potassium thiocyanate, a white precipitate of copper thiocyanate is formed instead of the usual reduction to the yellow or red precipitate of cuprous oxide.

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[1] Benedict's Quantitative Solution:

Copper sulphate (crystallized)	18 gm.
Sodium carbonate (crystallized)	200 gm.
Sodium citrate	200 gm.
Potassium thiocyanate	125 gm.
Potassium ferrocyanide, 2 per cent solution	5 cc.
Distilled water	1000 cc.

With heat dissolve carbonate citrate and thiocyanate in enough water to make 800 cc. of mixture. Dissolve copper sulphate separately in 100 cc. of water and pour the solution slowly into the other with constant stirring. Add ferrocyanide solution. Cool and dilute exactly to 1 liter. Five cubic centimeters is reduced by 10 mgm. of glucose.

Anhydrous Sodium Carbonate. Talcum:

Three test-tubes, 6 by ¾ inch; centrifuge tube, 5 cc.; and 1-cc. pipet or glass syringes.

The Test.—Use the qualitative test first to find out approximately the amount of sugar present.

Place in a test-tube 5 cc. of Benedict's quantitative solution, anhydrous sodium carbonate enough to fill an ordinary centrifuge tube to where the expansion ceases and a pinch of talcum to prevent bumping. Add urine from a 1-cc. pipet or hypodermic syringe, 0.25 cc. at a time and boil for one-half minute between each amount. The end-point is reached when all the greenish-blue disappears, leaving a creamy color. If 1 cc. of urine is required, it contains 1 per cent of sugar. If 0.5 cc. is required, it contains 2 per cent, etc. It is frequently difficult to determine the end-point if the sugar is below 0.5 per cent.

For accurate estimation do a second test adding at half-minute intervals, *drop by drop,* the last 0.25 cc. of urine required to whiten the green solution. The number of cubic centimeters of urine used, divided into 1 is the percentage of sugar in the sample. (If there is over 4 per cent of sugar, dilute the urine 1 in 6, estimate the sugar percentage and multiply the result by 6):

Example.—With 1.3 cc. of urine required the percentage of sugar would be $\frac{1}{1.3}$ which is 0.75 per cent.

There is frequently no sugar in the morning urine of the diabetic while several hours after a meal it may contain 2 to 5 per cent. The quantitative estimation of a single specimen is therefore of little value. The estimation of the percentage on a mixed twenty-four-hour specimen is very valuable. Collect the total quantity for twenty-four hours, measure it, and after mixing it well estimate the sugar percentage on a small sample of it.

Example.—If the total twenty-four urine was 1800 cc. (60 ounces) and the mixed sample of it contained 2.5 per cent of sugar, the total twenty-four-hour excretion would be 45 gm. (1¼ ounces) of glucose. For interpretation, see page 278.

Sugar Percentage by Fermentation.

The results by this method are only approximate and it should be used only as a makeshift when Benedict's quantitative solution is not available.

The Test.—If the urine is not acid in reaction add a few drops of 10 per cent acetic to render it slightly acid.

Powder ½ inch cube of yeast and add it to 50 cc. of urine. Take the specific gravity carefully and record it together with the temperature of the urine. Mark the height of the fluid and keep warm (80° to 110° F.) for two or three hours. Then test the urine for the presence of sugar. If none is present fermentation is complete. Cool to the temperature at which the specific gravity was originally taken and carefully take the specific gravity. The difference between the specific gravity before and after fermentation multiplied by 234 gives the percentage of sugar. (Roberts.)

At lower temperatures more time may be required to complete the fermentation.

Benedict's Picrate Method.—(Adopted by the committee on urinary impairments of the association of Life Insurance Medical Directors of America.)

Measure 1 cc. of centrifuged urine into a test-tube similar in size to the standards and graduated at 25 cc. Add 3 cc. of picric acid solution (2 gm. of pure dry picric acid per liter) and 0.5 cc. of 5 per cent sodium hydroxide. Add next, 5 drops of 50 per cent acetone solution (prepared fresh each day by diluting acetone with an equal volume of water) and place the tube promptly in a boiling water-bath. In twelve minutes remove the tube, cool and dilute the contents to 25 cc. Compare this colored solution with permanent standards in tubes of the same internal diameter and estimate the amount of sugar. Five standards (manufactured by Fales Chemical Company) 0.1, 0.2, 0.3, 0.4 and 0.5 per cent are used. In case more than 0.5 per cent sugar is present dilute the urine 1 in 10 and repeat the determination. The value of the standard most closely matched multiplied by 10 gives the desired value. Under 0.25 per cent is not considered pathological.

Remarks.—This test shares the shortcomings of the copper reduction tests in that it is not specific for glucose. Highly concentrated urines containing no glucose but much urate and phosphate sediment will show up to 0.2 per cent reaction for glucose. It requires more reagents and time than Benedict's copper sulphate test and its only advantage is in the demonstration of small amounts of sugar from 0.2 to 0.5 per cent. These small percentages are not readily demonstrable by Benedict's copper sulphate quantitative test.

Tests for Acetone Bodies in the Urine (Ketonuria).—No attempt is made to use separate tests for acetone and diacetic (acetoacetic) acid. These substances occur together and have the same significance. In ketonuria freshly passed urine usually contains more diacetic acid but on standing it slowly changes over into acetone. The sodium nitroprusside test which was formerly thought to be specific for acetone is found to be much more sensitive for diacetic acid. First do a sodium nitroprusside test and if it be positive also try the ferric chloride test which will react only to large amounts of diacetic acid.

Sodium Nitroprusside Test for Acetone Bodies. (Lange.)—Acidify 5 cc. of urine with a few drops of 10 per cent acetic acid. Add a small grape-seed-sized crystal of sodium nitroprusside and shake to dissolve. Tilt the tube and carefully overlay the solution with ammonium hydroxide.

Negative.—Yellow or brownish at point of contact.

Positive.—A purple color at the point of contact. With large amounts it shows immediately; with small amounts, after one-half minute. This test will show a faint positive reaction to 0.004 per cent diacetic or 0.1 per cent acetone.

Ferric Chloride Test for Diacetic Acid. (Gerhardt's.)—to 10 cc. of fresh urine in a test-tube add 10 per cent aqueous solution of ferric chloride until the precipitate clears.

Negative.—Buff to deep yellow with normal urine.

Positive.—A deep red color when 0.07 per cent diacetic acid is present. If in doubt do the test on a normal urine and compare results.

Caution.—A confusing purple color is produced by the urine of normal patients who have taken aspirin, antipyrin, phenacetin, salicylates, salol and sodium bicarbonate. The reaction is still maintained after boiling the urine if it be due to drugs whereas boiling for three to five minutes converts the diacetic acid into acetone which does not react with ferric chloride.

Interpretation.—Acetone bodies in the urine is known as ketonuria. It occurs in diabetic acidosis of young and old and if the acetone bodies be present in great concentration, coma develops. A mild ketonuria develops after ether or chloroform anesthetics. This may be corrected by sufficient water and sugar intake. For the diabetics who cannot metabolize sugar, insulin is necessary in addition to water and sugar. The acetone bodies are made up of acetone, diacetic and β oxybutyric acid. The diacetic acid usually makes three-quarters of the whole. Both acetone and β oxybutyric may be formed from diacetic acid.

Ketonuria, which is usually regarded as a definite sign of acidosis because of studies in diabetes, should be regarded as a sign of imperfect fat metabolism resulting from carbohydrate starvation. The ketonuria of diabetes is the cause of acidosis but it is wrong to suppose that every ketonuria is accompanied by an acidosis. It is possible especially in the ketosis of childhood which is frequently accompanied by vomiting to have a definite alkalosis as shown by an increase in the alkali reserve and increased pH of the blood together with acetone bodies in a urine of alkaline reaction. Sufficient fluid and sugar intake usually corrects this state.

Chlorides in Urine.

Quantitative Test.—Remove any albumin present by boiling, adding a few drops of acetic acid and filtering. Dilute 1 cc. of urine with 20 cc. of distilled or chloride-free water in a test-tube. Add a few drops of concentrated nitric acid to acidify and 10 to 15 drops of 10 per cent silver nitrate solution. A white opaque precipitate of silver chloride forms with the amount of sodium chloride normally present. There may be only a slight milky turbidity when the chloride output is diminished. By doing a test on a normal urine as a control one can readily tell whether the chloride output is normal, increased or diminished. The chloride intake should be considered.

Quantitative Estimation, Simplified Volhard Method.—Solutions.—1. Standard silver nitrate solution. (Standard silver nitrate solution—silver nitrate chemically pure anhydrous, crystallized, 29.055 gm.; nitric acid (25 per cent) solution, 900 cc.; ammonium ferric alum (cold saturated solution), 50 cc.; distilled water to 1000 cc.).

2. Ammonium sulpho- or thiocyanate solution. (Ammonium sulpho- or thiocyanate 7 gm., distilled water 1000 cc.).

Standardize several hundred cubic centimeters of the above concentrated solution by diluting it with distilled water until 20 cc. will produce a red color when mixed with 10 cc. of the standard silver nitrate solution. If 16 cc. of the solution No. 2 produce a red color when mixed with 10 cc. of solution No. 1 it will be necessary to add 4 cc. of every 16 cc. of solution No. 2.

To 16 cc. solution No. 2 add 4 cc. of water.

To 1 cc. solution No. 2 add $\frac{4}{16}$ cc. of water.

To 200 cc. solution No. 2 add $\frac{4}{16} \times 200 = 50$ cc. of water.

Therefore add 50 cc. of water to 200 cc. of solution No. 2. Check the solutions at weekly intervals.

3. One ounce of 10 per cent potassium permanganate.

4. Fifty cubic centimeter graduated cylinder with glass stopper.

The Test.—Collect a twenty-four-hour specimen of urine from a mixed sample, place 5 cc. in the 50-cc. graduated cylinder, add 10 cc. of the standard silver nitrate solution. Mix by inverting. Should a reddish color appear add several drops of the 10 per cent permanganate solution.

After five minutes add the standard thiocyanate solution a cubic centimeter at a time, shaking after each addition. Continue to add small amounts until a permanent reddish-brown color appears.

The difference between 35 cc. and the height of the fluid in the cylinder when the end-point is reached is the number of grams of chloride per liter of urine.

The solutions are so prepared that if no chloride be present the reddish-brown end-point will be reached at 35 cc. Every gram of chloride per liter of urine will take up 1 cc. of the standard silver solution.

Example.—If the fluid turned a permanent reddish-brown at 31 cc., $35 - 31 = 4$, which would be the grams per liter of urine. Calculate the twenty-four-hour output.

Interpretation.—The normal urinary chlorides is 10 to 15 gm. in twenty-four hours. It is greatly affected by the sodium chloride intake being much diminished in salt-poor diets or in starvation. The intake of sodium chloride should be regulated for the test. In chronic parenchymatous nephritis with lesions of the urinary tubules the chloride excretion is poor. Edema is frequently present. Inflammatory states where there is accumulation of an exudate as in pneumonia there is a marked diminution in the urinary chlorides while the exudate is forming and a great increase when the exudate is resolving.

Test for Indican.

Apparatus and Reagents.—Test-tubes; Obermeyer's reagent (0.4 per cent ferric chloride in strong HCl 4 ounces); chloroform 2 ounces.

The Test.—Mix 1 inch of urine in a test-tube with an equal volume of Obermeyer's reagent. Add ½ inch of chloroform and shake gently. On settling the chloroform becomes tinged blue if indican is present. The depth of color indicates the comparative amount of indican if the same proportions of urine and reagent are always used. A normal control should be done.

NOTE.—Iodides, guaiacol, urotropin when taken, and formalin or bile in the urine interfere with the reaction.

Interpretation.—Indican in the urine is increased (1) when the diet is rich in protein. (2) In obstruction of the small intestine. (3) Intestinal putrefaction occurring in chronic constipation. (4) Large collections of pus in the body, peritonitis, empyema and large abscesses.

Test for Diazo.

Reagents.—1. Sulphanilic acid, 1 gm.; hydrochloric acid concentrated, 10 cc.,; water, 200 cc.

2. Strong ammonia.

3. Sodium nitrite, 0.5 per cent, freshly prepared (1 gm. tablet in 13 cc. of water).

The Test.—Dissolve 1 sodium nitrite tablet in 3 cc. of water. Mix 100 parts of the sulphanilic solution with 1 part of the nitrite solution. In a test-tube take equal parts of this mixture and the urine and overlay with a little ammonia. If the liquid becomes a deep red color at the junction of the two fluids and upon shaking a pink color be imparted to the foam it is *positive.* Any trace of yellow or orange denotes a negative reaction. Drugs such as iodides, salol, atopan, tannic acid and the opiates interfere with the reaction.

Interpretation.—The reaction is present from the fourth to the fourteenth day in typhoid. A negative reaction is of more value against the disease than a positive reaction is in favor of it. It is of more import to distinguish between a relapse and a complication. The reaction is present in measles, but not usually in German measles. It is very constantly present in tuberculous disease which is advancing rapidly. Miliary tuberculosis may simulate typhoid clinically.

Test for Bile Pigments in the Urine.

1. **Foam Test.**—Bile in the urine gives it a yellowish-green or brownish color. On shaking, a copious yellow foam is formed. A yellow color of the foam is proof of bile being present. This is a reliable test but will not show minute amounts.

2. **Oxidation Tests for Bile.**—To 5 cc. of urine in a test-tube add an equal amount of concentrated hydrochloric acid. After mixing add a few drops of hydrogen peroxide. A green color which develops after

a minute denotes the presence of bile. A few drops of Obermeyer's reagent will also give a green color to urine if bile is present.

The surface tension test for bile acids is not definite enough for a clinical test. Pulverized sulphur floats in cool normal urine but sinks if bile acids are present in 1 in 100,000 amounts. Frequently some of the sulphur will sink in normal specimens having a high specific gravity.

Interpretation.—The bile that appears in the urine is usually due to obstruction of the biliary ducts by calculus, colangitis or tumor. Occasionally it is due to a hepatitis. The hemolytic jaundice produced by hemolytic diseases does *not* give bile in the urine although it imparts a dark color, due to urobilin. This is one of the most practical and certain methods of differentiating an obstructive from a hemolytic jaundice. The kidney cannot excrete bile unless it has been separated from the blood plasma by the liver.

Bile in the urine is an earlier sign of biliary disorder than jaundice. In the severe obstructive cases bile in the urine appears at least a day before jaundice of the sclerotics. In cases of partial biliary obstruction or toxic hepatitis, bile may appear in the urine but no definite jaundice occurs.

Test for Urobilin in the Urine.—A large amount of urobilin in the urine gives it a dark-brown color. One would suspect a slight amount of bile but on shaking it there is no yellow tinge to the foam. It is excreted as urobilinogen and by the action of light for three or four hours it changes to urobilin.

Reagents and Apparatus.

Lugol's iodine solution, 2 ounces.
Saturated alcoholic solution of zinc acetate, 8 ounces.
Filter paper and funnel.
Ten per cent aqueous solution of calcium chloride.

The Test.—If bile is present it may be removed by first adding 2 cc. of 10 per cent calcium chloride to 10 cc. of urine.

To 10 cc. of urine add a few drops of Lugol's solution to transform all the urobilinogen into urobilin. Mix with 10 cc. of the saturated alcoholic solution of zinc acetate and filter. The filtrate will show a green fluorescence if urobilin be present. This is best seen against a black background with a bright light coming from one side. If positive repeat the test using urine diluted 1 in 10, 20, 50 and 100. Test on several days.

Interpretation.—A fluorescence occurring in a dilution greater than 1 in 20 is considered pathological. An increase of urobilin is present in the urine when there is excessive blood destruction and also when there is a partial damage to the parenchyma of the liver. It is absent if no bile passes into the intestine as in complete obstruction.

Urobilin is supposed to be formed by the action of intestinal bacteria on bile excreted from the liver. It is then absorbed and the liver again turns it into bilirubin.

Hemolytic diseases will produce an increase in the output of bile and more urobilin will be formed in the intestine than usual. When this excess is absorbed part of it goes to the liver to form bilirubin and the rest is excreted by the kidney. When no unusual hemolysis exists and the bile pigment flows normally into the intestines if the liver is partially damaged it cannot convert all the urobilin that is absorbed from the intestine and so the extra amount in the blood stream is excreted by the kidneys. So a urobilin increase may signify liver damage such as cirrhosis when there is no excessive hemolysis. If all the bile is dammed back from the bowel by calculus, infection and new growths, no urobilin can be formed by the bacteria in the intestine and none will be found in the urine.

Examination of Urine for Tubercle Bacilli.—The urine has an acid reaction and moderate amounts of pus but no pyogenic bacteria are present. A small amount of albumin is common in active tuberculosis.

Method of Collecting the Urine.—A clean bottle should be supplied. A catheter should be used to obtain the specimens from females. Males, after discarding the first few ounces, collect the remainder in the bottle.

Examine three or four specimens preferably those passed in the forenoon after the patient has moved about.

The examination should be done before the urine becomes stale and non-pathogenic bacteria multiply to obscure any tubercle bacilli. With each examination at least 100 cc. of the urine should be centrifuged.

The Test.—The urine is first centrifuged one minute to precipitate the crystals and most of the pus cells. A few pus cells and most of the bacilli remain suspended in the fluid.

The supernatant fluid is now removed and mixed with an equal amount of 50 per cent alcohol which renders the specific gravity of the fluid less than the bacilli.[1] This fluid is now centrifuged at high speed for one-half hour, the supernatant fluid discarded and the sediment transferred to a glass slide by a pipet and fixed with a flame. It is best to add a drop of blood serum or egg albumen fixative. (Equal parts of egg-white and glycerin with 1 per cent sodium salicylate as a preservative.) Stain with carbol fuchsin as outlined for sputum on page 224.

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[1] Jones: Jour. Am. Med. Assn., 1924, **83**, 1917.

The bacilli when present appear in large clumps rather than isolated bacilli as in the sputum. One may examine for ten minutes without seeing a single tubercle bacillus and then suddenly come upon a large clump with scores of tubercle bacilli heaped together. In no other examination does painstaking search of repeated specimens yield such valuable results which may lead to the diagnosis of renal tuberculosis.

Interpretation.—A persistent pyuria, in an acid urine containing no Bacillus coli or cocci and occasionally blood is highly suspicious of renal tuberculosis.

If clumps of tubercle are found microscopically in the sediment of specimens collected under special precautions a definite diagnosis may be made of urogenital tuberculosis in the male or urinary tuberculosis in the female. Cystoscopic examination will determine whether the infection is bilateral or unilateral, also the function of each kidney will be established.

It has recently been asserted that a healthy kidney may excrete tubercle bacilli in patients who have active lesions in the lungs, bones or joints. This secretory bacilluria is so scanty that it is detected only when very large amounts of urine are examined and the lung or bone lesions are advanced. There is no pus and the bacilli are not in clumps. The presence of pus in the urine indicates the urogenital tract as the site of the tuberculous process and the bacilli are in clumps containing great numbers. Even with renal lesions the tubercle bacilli do not appear at all times because the infected area may be walled off. In as high as 18 per cent of renal tuberculosis proven at operation, examination of the urine and inoculation into guinea-pigs failed to demonstrate the presence of the bacillus.[1]

Guinea-pig inoculation is regarded as our most sensitive test, particularly when it is repeated, but infection will not develop if the bacilli are dead or if less than 10 bacilli are inoculated (Calmette). A few guinea-pigs have a natural high immunity to tuberculosis and cannot be infected. Tuberculosis of the kidney was found in every case at the Mayo Clinic where there was a positive finding of tubercle bacilli on microscopic examination or guinea-pig inoculation.[2]

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[1] Morse and Braasch: Jour. Urol., 1927, **17**, 287.
[2] Morse and Braasch: Ibid., 1929, **17**, 287.

Cystoscopic examination and urography are of great value in the diagnosis of renal tuberculosis.

Method of Guinea-pig Inoculation for Tuberculosis.—With suspicious symptoms of genito-urinary tuberculosis a guinea-pig may be inoculated with the urinary sediment either to confirm the finding of microscopic examination or to detect the presence of bacilli when they are too few to be readily discovered under the microscope. Use fresh urine and inoculate 1 or 2 cc. of the sediment as collected for microscopic examination. Use a large 20-gauge hypodermic needle and inject under the loose skin of the groin. It is best to use two guinea-pigs in case one dies from infection by pyogenic organisms. Weigh the animals weekly and if they start to lose weight after the third week kill them and examine carefully for characteristic yellowish-gray tubercles in the inguinal and retroperitoneal lymph glands. They may also be present in the peritoneum, spleen and liver. A continued gain in weight is regarded as a negative result.

Culture for Colon Bacilli.—*Apparatus.*—One in 1000 bichloride solution, absorbent cotton, female catheter, sterile receptacle.

Obtaining the Specimen.—*From Males.*—Sponge off the urethral orifice with bichloride wipes for one-half minute. The patient should then urinate a small amount to flush out the urethra and passes the second part into a sterile receptacle.

From Females.—Also sponge off the urethral orifice with bichloride wipes, pass a catheter and collect the urine in a sterile container.

The Test.—Examine a smear for bacteria. If many be present plate out thinly on media and search for minute streptococcus colonies. If few or none are seen a few drops of urine should be cultured. A profuse growth of Gram-negative rod-shaped bacilli with a fecal odor indicate a colon culture. This can be confirmed by subculturing on glucose and lactose. Colon bacilli produce acid and gas on both sugars. Typhoid produces acid on glucose only. Only in the presence of symptoms is a colon infection somewhere along the genito-urinary tract indicated and a vaccine may be prepared from this culture.

Biological Test for Pregnancy. (Ascheim and Zondek.)

Principle.—In pregnancy there is a marked overproduction and excretion in the urine of the hormone from the anterior lobe of the pituitary gland. This hormone produces marked hyperplasia of the ovaries and if urine containing it be injected into immature female mice typical ovarian changes may be noted.

The Test.—Female white mice three to six weeks old and weighing 10 to 15 gm. should be used.

Inject subcutaneously three times daily for three days 0.3 cc. (5 minims) of fresh urine from morning specimens. Two days after the last injection do a necropsy on the mice, examining the pelvic organs carefully. A positive finding consists in:

1. *Swelling.*—The ovary is three times the normal size.
2. *Congestion.*—Gives it a pinkish appearance and subcutaneous hemorrhages may be seen.
3. *The ripening ovarian follicles* may be seen as yellow spots. This is in contrast to the normal ovaries, which are small pale bodies.

Urine from a non-pregnant individual should be injected into a second mouse as a control test.

While this test is comparatively a new one all reports indicate a high percentage of correct results. It gives positive reactions as early as the third week of pregnancy and up to full term.[1]

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[1] Louria and Rozenzweig: Jour. Am. Med. Assn., 1928, **91**, 1928.

CHAPTER X.

EXAMINATION OF THE FECES.

FUNCTIONAL TESTS OF THE GASTRO-INTESTINAL TRACT

Gastro-intestinal Motility.—The time taken for food to pass through the gastro-intestinal tract may be determined by giving a 10-grain capsule of carmine or charcoal during a meal.

The stools should normally become colored red in twenty-four to forty-eight hours and all the red color should be eliminated within forty-eight to seventy-two hours after taking the carmine capsule.

The bowels do not empty properly if the feces are colored with the carmine after seventy-two hours. This is a simple, economic method and should be in more common use. Those who do not show a normal evacuation of the red pigment require further examination to determine the cause of the stasis. Rectal examination or tests on the resting gastric contents and the x-ray are useful methods for further investigation.

Collection of Feces.—Supply the patient with a quart-size fruit jar which has a close-fitting cover. It should contain ½ ounce of formalin to destroy the disagreeable odor which is the greatest objection to the examination of feces. It is necessary to omit the formalin only when examination for occult blood, pathogenic bacteria or amœba is required. Toluene does not interfere with the reaction for occult blood.

Gross Inspection.—It is very important to note the color and form of the stool. Also look for mucus, pus or blood.

Color.—The normal color of feces varies from yellow to brown. If the color is lighter than normal the usual cause is obstruction of the common bile duct. Poor digestion of the fatty particles of the food due to inadequacy of the pancreatic lipase is sometimes a cause of a pale-colored, porridgy stool.

Vegetables with reddish pigment, such as beets, and chemicals such as carmine, will cause red stools. Fresh red blood most often comes from internal hemorrhoids, anal fissure or carcinoma of the rectum. When the blood has a brownish tinge it comes from higher up. Blood from the stomach or duodenum is black. It is necessary before ascribing a tar-black stool to intestinal or gastric hemorrhage to rule out the ingestion of iron or bismuth which also render the stool black. Spinach contains enough iron to produce this reaction.

Form.—Normal stools should be semi-formed with an outline resembling a small banana. Undigested food particles may be seen. The skin of apples, pears, peaches and other fruits, also cabbage and coarse greens appear in the feces normally. When pieces of meat and boiled vegetables, apart from those of coarse tough cellulose texture, appear, the digestive process is deficient. *The totally unformed stool indicates a lack of proper absorption by the large intestine.* This may be the result of a quickly emptying achlorhydric stomach, hyperirritability of the intestine, a diffuse colitis or local ulcers in the rectum, which cause exaggerated reflex motility of the whole colon.

If the stools are narrow and ribbon-like the most likely cause is spasm. It may be a rectal stricture, the result of an ulcer or carcinoma. Especially is the latter suspected if the stool is streaked with blood. Small round masses of feces (scybalæ) are the result of a spastic condition of the colon. Masses of feces are held up by colonic spasm until most of the moisture in them is absorbed, leaving a hard round fecal mass. Patients with this condition have incomplete bowel movements. Large firm masses of feces are the result of an atonic condition, usually in the sigmoid, where the fecal matter accumulates over a long period of time before sufficient stimulation is produced on the bowel wall to expel it.

Mucus and pus in large amounts indicate colitis or enteritis. In the stools of acute diarrheas the mucus is intimately mixed with the fecal material and may be detected by passing a glass rod or platinum wire through the mass and noting the mucoid character of it. In the more solid stools it occurs as large shreds usually on the surface. Mucus coming away periodically either in shreds or a complete cast of the bowel lumen is present in the clinical condition known as mucous colitis. Pus is not present in this condition. Pus is usually found associated with mucus in the ordinary inflammatory conditions. Microscopic examination of the mucus will therefore differentiate mucous colitis from inflammatory colitis.

Intestinal parasites or segments of them may appear in the feces and often resemble shreds of mucus. This will be more fully discussed under the section on Intestinal Parasites. For more detailed information of the physical characteristics of stools, a book on Clinical Diagnosis should be consulted.

Chemical Tests.—The chemical tests most frequently made are for blood and bile. The test for occult blood is by far the most important.

TEST FOR OCCULT BLOOD.

This test is of great clinical importance. The reliability of the reagent can be checked by adding it to a drop of blood diluted in a test-tube full of water. Almost as important is to use exactly 25 mgm. of benzidine which in 5 cc. of acetic acid will make a 0.5 per cent solution. This gives the test the proper degree of sensitiveness for clinical use.[1]

Many authorities give rigorous precautions to the patient to avoid drugs, meat and green vegetables, also not to brush the teeth vigorously to avoid making the gums bleed. If the above technique is used practically none of these restrictions are necessary. Taking a large amount of under-done meat or *bone-marrow* will sometimes give a weak delayed reaction.[2] To place a patient on a restricted milk diet defeats the object of the test because very bland food checks the bleeding of ulcers.

Obtaining the Specimen.—Supply the patient with two small wide-mouthed bottles each of several ounces capacity and well corked. Formalin if used as a deodorant will give a false positive reaction and carbolic acid will give a negative reaction when blood is present. Toluene does not interfere with this reaction and a dram may be used as a deodorant when desired. Ask the patient to obtain a mass of stool about a cubic inch, on two successive days.

The Test.—Keep on hand carefully prepared benzidine powders, each containing benzidine, chemically pure, 25 mgm.; barium peroxide, 0.2 gm. and put up in waxed papers; also a 50 per cent aqueous solution of acetic acid.

Just before doing the test dissolve the contents of one of the waxed papers in 5 cc. of the 50 per cent acetic acid. The solution will remain good only for one-half hour. Then, by means of a wooden spatula, smear a small amount of the stool over a glass slide or post-card and allow a few drops of the benzidine acetic acid solution to fall on the smear. A blue or bluish-green color develops within a minute if the test is positive and the reaction is graded according to the depth of the color and the time it takes to develop.

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[1] Gregersen, J. R.: Arch. f. Verdauungskr., 1919, **25**, 3.

[2] Ogilvie: Brit. Med. Jour., 1927, i, 755.

A deep blue within three seconds indicates over 5 per cent of blood.

A definite pale blue within twelve to fifteen seconds indicates 1 to 5 per cent of blood.

A pale blue or green within thirty seconds indicates less than 1 per cent of blood.

The eating of under-done meat will sometimes give a pale blue after thirty seconds.

Interpretation.—In a positive benzidine reaction on the stool, exclude the nose and mouth as a source of *gross* bleeding by examining for gingivitis, or bloodclots in the nose. The rectum should also be examined for fissure or bleeding hemorrhoids. The blood will be on the surface of the fecal masses when the bleeding is from the anus.

A positive reaction indicates ulceration of the gastro-intestinal tract when the above sources are checked and injury by swallowing caustics or sharp articles excluded.

In conjunction with symptoms suggestive of gastric or duodenal ulcer the finding of blood in the stool confirms the diagnosis. The bleeding is intermittent however and occurs in only 75 per cent of benign ulcers. The disappearance of blood is an indication that the ulcer is healing.

In carcinoma of the gastro-intestinal tract there is a positive reaction for occult blood in the feces in over 90 per cent of cases. The negative reactions usually occur with malignancy of the esophagus or pylorus. In carcinoma of the esophagus the occult blood reaction may be negative in 40 per cent of cases.[1] Scirrhous carcinoma of the pylorus rarely produces intestinal bleeding but the gastric retention and vomiting which soon occur in this condition, facilitate the diagnosis.

In gastro-intestinal cancer not at the esophagus or pylorus the occult blood reaction is almost constantly positive. It occurs as soon as there is any ulceration, which is quite early in the clinical course of the disease. The Gregersen technique which is outlined above is quite reliable and if performed repeatedly it may be the first sign of malignancy. The procedure is simple and the cost is insignificant compared with x-ray investigation.

A more remote cause of occult blood in the stool is cirrhosis of the liver, giving rise to esophageal varicosities, which may bleed into the stomach.

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[1] Meulengracht and Jensen: Jour. Am. Med. Assn., 1929, **92**, 697.

SCHMIDT'S TEST FOR BILIARY PIGMENTS IN THE FECES.

Reagent.—Mercuric chloride saturated aqueous solution:

Mercuric chloride	6.3 gm.	or	101 gr.
Distilled water	100.0 cc.		4 oz.

Large test-tube.

The Test.—Place a mass of feces about ½ inch in diameter in a test-tube. Add three or four times its volume of saturated mercuric chloride solution and mix with a glass rod or several wooden applicators.

Red denotes hydrobilirubin (urobilin) and green denotes bilirubin. The color appears in less than an hour but may require several hours when only small amounts of bile are present.

Interpretation.—The normal coloring matter of feces is hydrobilirubin (urobilin). It is formed by the action of the intestinal bacteria on the bile, which comes from the liver chiefly as bilirubin. If the bowel passes contents along rapidly the intestinal bacteria do not have time to convert the bilirubin into urobilin.

Obstruction of the common bile duct from a calculus is rarely constant or complete whereas obstruction from carcinoma is complete and there is total absence of bile in the stool. The chemical test is essential to determine whether small amounts of bile are present or not. A diminished amount of bile in the stool as well as its total absence, will give rise to the large, undigested, pale-colored stool. Jaundice and bile in the urine will be present with partial as well as complete biliary obstruction.

MICROSCOPIC EXAMINATION OF FECES.

Microscopic examination of a small particle of fresh feces mixed with an equal amount of water on a glass slide and covered with a cover-glass will reveal many structures varying greatly in form and color. The film should be thin enough to read print through it. For the most part the objects are bacteria; vegetable fibers, spirals or cells; triple phosphate crystals; connective-tissue strands and muscle fibers; epithelial cells and leucocytes. Occasionally amœba and flagellates are present. Pathologically there may be a great increase in the leucocytes. Red blood cells, pathogenic bacteria and protozoa or the ova of animal parasites may be present. A drop of Lugol's iodine added to the margin of the cover-glass will determine whether starch digestion is complete. After normal digestion there is no blue staining starch granules. If cross striations remain on the meat fibers, there is deficient digestion of protein. If an excess of these foods is taken undigested portions will be found in the stool even when digestion is adequate. Moreover after normal meals a few parts of the stool may show imperfectly digested food while other parts show satisfactory digestion. One should therefore not depend too much on the microscopic appearance of a single particle.

INTESTINAL WORMS.

Suspecting the presence of intestinal parasites is the first essential in their detection. A positive diagnosis of intestinal worms can be made only on finding part or all of the parasite in the stool or by finding their ova microscopically. The parasites appear only at infrequent intervals but the eggs are present at all times. The logical examination therefore is to search the stools for the eggs and this is easily done. No examination of an anemic patient is complete without searching the stools for parasitic ova and the diagnosis of "worms" is not justifiable without finding ova or remnants of parasites in the stool.

Examination for Ova.—Collection of Feces.—Do not order a purge before collecting the specimen. Collect a mass of feces somewhat larger than a hen's egg in a quart-sized wide-mouthed fruit sealer containing an ounce of formalin and a pint of water. The formalin destroys the disagreeable odor which is the greatest objection to the examination of feces and the water tends to emulsify the mass and render the isolation of the ova more certain.

The Test.—A concentration method is best. It depends on the rapid settling of the ova in a watery solution. The following is the least objectionable and most easily performed technique.

Break up any firm masses with a spatula. Cover the jar tightly and shake vigorously for one-half minute. Allow two minutes for the sediment, which consists of fecal particles and ova, to settle. Pour off the supernatant fluid to get rid of the bacterial content of the stool and again fill the jar two-thirds full with fresh water. Cover and shake as before. Then strain the contents through a layer of coarse gauze over a funnel into another glass jar. Most of the ova will go through the gauze but the large particles of feces will be held back. The ova are heavy and they settle as a brown sediment in less than two minutes. Tilt the bottle so the sediment will settle in one corner. Transfer a drop of this sediment by means of a pipet to a glass slide. Put on a cover-glass and examine under the low-power lens of the microscope with subdued light.

When ova are present no doubt exists even in the mind of one unacquainted with their appearance. They appear as numerous large bodies of uniform size and shape. (See Fig. 87.) The outer membrane is thin and different material makes up the contents. There are no other particles in the feces so unvaried, and if one is in doubt whether or not a few irregular, roundish bodies are ova it is almost certain they are not.

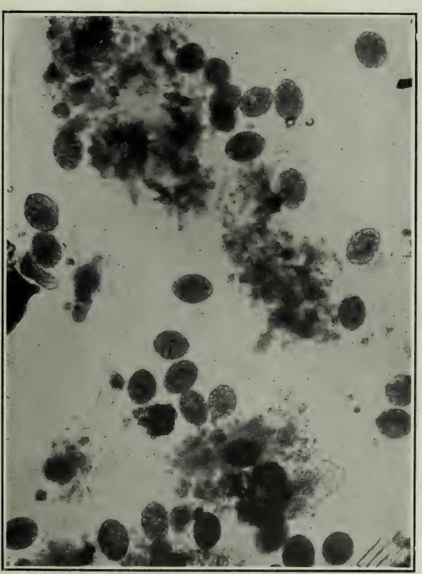

FIG. 87.—Ova of fish tapeworm as seen with the low-power lens and × 10 ocular. (× 100.)

This method will isolate the ova of any parasites with ease and so assure the diagnosis. For the immediate treatment it does not much matter what type of worm is present but most of the species can be differentiated from plates illustrating their typical ova.

The Removal and Examination of Intestinal Parasites.

Preparation of Patient.—Omit the evening meal and give a saline purge instead, followed by the anthelmintic in the morning. Abundance of fluids but no food is taken during the test. For roundworms or pinworms the remedy is usually oil of chenopodium, 3 doses at two-hour intervals each, 1 minim for every year the patient is old, up to 15. For tapeworm 2 doses, an hour apart, each dose containing, for an adult, a dram of the liquid extract of male fern made into an emulsion. The vermifuge should be followed in two hours by ½ ounce of magnesium sulphate as a purge. Castor oil should not be given because it aids in the absorption of the male fern which is quite toxic. Two hours after the saline complete the treatment by giving a large soapsuds enema.

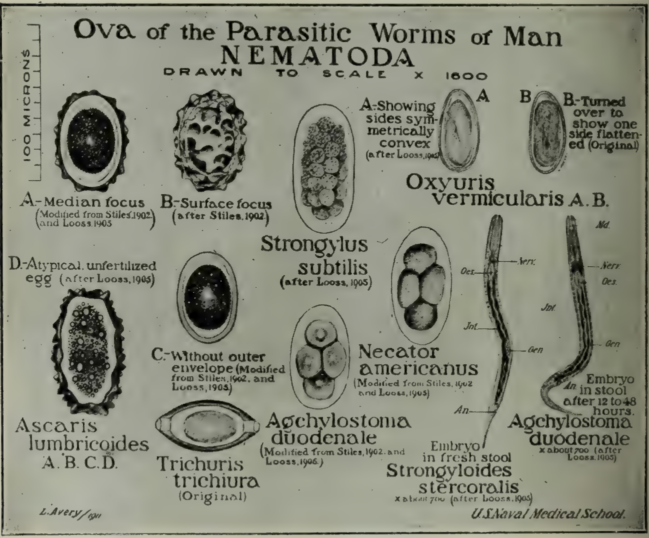
Ova of the Parasitic Worms of Man
NEMATODA
DRAWN TO SCALE X 1600
100 MICRONS
A-Median focus (Modified from Stiles 1902 and Looss 1905)
B-Surface focus (after Stiles 1902)
D-Atypical unfertilized egg (after Looss, 1905)
C-Without outer envelope (Modified from Stiles, 1902, and Looss, 1905)
Ascaris lumbricoides A. B. C. D.
Trichuris trichiura (Original)
Strongylus subtilis (after Looss, 1905)
A-Showing sides symmetrically convex (after Looss, 1905)
A
B
B-Turned over to show one side flattened (Original)
Oxyuris vermicularis A. B.
Necator americanus (Modified from Stiles, 1902 and Looss, 1905)
Agchylostoma duodenale (Modified from Stiles, 1902 and Looss, 1906.)
Nerv.
Oes.
Jnt.
Gen
An.
Md.
Embryo in fresh stool
Embryo in stool after 12 to 48 hours.
Strongyloides stercoralis x about 700 (after Looss, 1905)
Agchylostoma duodenale x about 700 (after Looss, 1905)
L. Avery/1911
U.S. Naval Medical School.

FIG. 88.—Ova of roundworms. (Stitt, Courtesy of P. Blakiston's Son & Co.)

Examine all the feces passed after the anthelmintic is administered until the last return of the enema. This is best accomplished by spreading two layers of medium gauze over the container and after the feces are passed into it gather up the corners of the gauze and allow a stream of water from a tap to pour over the sac. This will wash away bacteria and smaller particles. If any parasite or segments are present they will be readily seen among the coarse particles remaining in the sac. No toilet paper should be placed with the stool. A thorough search should be made for the head of the parasite. If in doubt about slender segments, the microscope will enable one to see whether or not the head is present.

INTESTINAL PARASITES.—There are two common classes of intestinal parasites in this country, the tapeworm or cestode and

the round worm or nematode. Common varieties of tapeworms are the fish tapeworm (Diphyllobothrium latum) originating from larva in fresh-water fish such as pike or pickerel, the pork tapeworm (Tænia solium) originating from larva in pork and the beef tapeworm (Tænia saginata) originating from larva in beef. The hydatid cyst passes its tapeworm stage in the dog and its cystic stage in the abdominal organs of man.

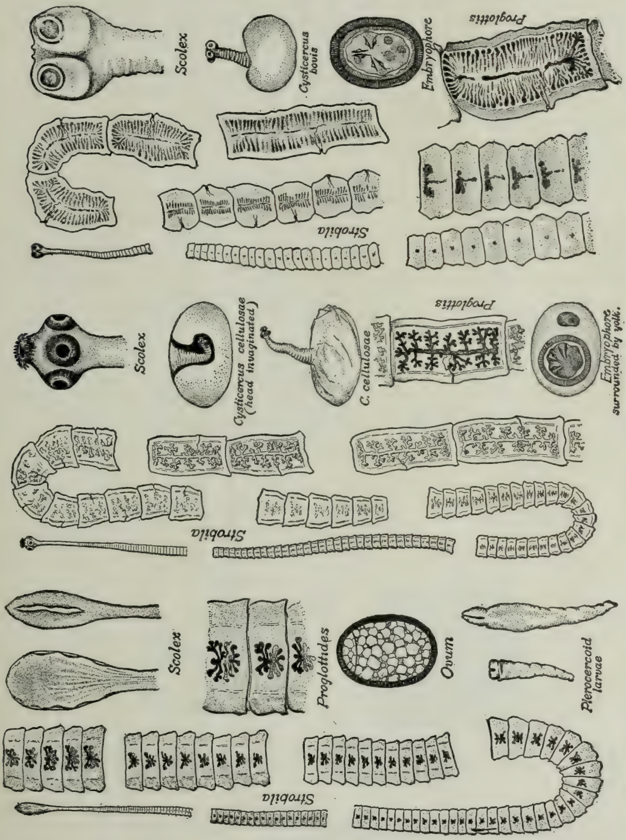
Scolex
Cysticercus bovis
Embryophore
Proglottis
Taenia saginata
Strobila
Scolex
Cysticercus cellulosae (head invaginated)
C. cellulosae
Proglottis
Embryophore surrounded by yolk.
Taenia solium
Strobila
Scolex
Proglottides
Ovum
Plerocercoid larvae
Diphyllobothrium latum
Strobila

FIG. 89.—Tapeworms that infest man. (Stitt, Courtesy of P. Blakiston's Son & Co.)

The Tapeworms.—Segments of the fish tapeworm are readily recognized because they are broader than they are long and the uterus forms a yellowish dot in the center. When a solitary worm is present it usually attains a length of from 15 to 25 feet. When many are present they are not so large. If the end segments degenerate rather than pass out they may cause an anemia somewhat like Addison's pernicious anemia. The head is about the size of the head of a common pin and attaches itself to the bowel wall.

The pork tapeworm may be from 5 to 10 feet in length and its segments are twice as long as they are wide. The uterus is branching with from seven to fourteen branches on each side. The head is smaller than the head of a pin. It has four sucking disks and a row of hooklets on the top.

The beef tapeworm is 10 to 20 feet in length. Its segments are twice as long as they are wide and each contains a uterus giving off twenty to thirty branches on each side. Its head is similar to that of the pork tapeworm but there is no row of hooklets. Segments, each containing many ova, pass out at frequent intervals.

Echinococcus cysts may be diagnosed on microscopic examination of the cyst fluid, by the presence of the typical hooklet either singly or in rows. The liver is the commonest site of this cyst formation.

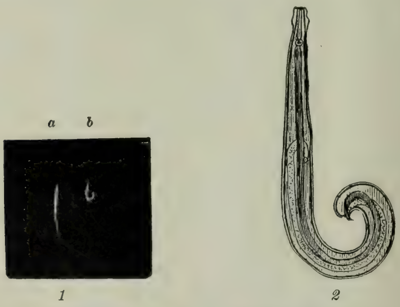

Fig. 90.—*1*, Oxyuris vermicularis: *a*, male; *b*, female; natural size. *2*, magnified. (Simon.)

Round Worms.—The two types of roundworms commonly found are the roundworm (Ascaris lumbricoides) and the pinworm (Oxyuris vermicularis).

The round worm is 6 to 16 inches long and less than a $\frac{1}{4}$ inch in diameter. They have a dull reddish color resembling ordinary earthworms but are larger and give off numerous ova. They inhabit the small intestine of children but may be present in the intestines of dogs, cats or hogs. Some of the parasites in their early stage of development wander through the body tissues but usually cause little disturbance except in the lung where they may cause hemorrhage.

Although the pinworm matures in the small intestine it generally lives in the cecum or rectum of young children where it causes a very intense itching. Occasionally the appendix contains many pinworms. The length is generally less than ¼ inch and they can be best found after an enema or purgative. The ova may adhere to the skin about the rectum and because of the intense itching they get under the finger-nails and so may be carried to the mouth to reinfect the individual. These parasites are capable of multiplying in the intestine without the introduction of eggs through the mouth however.

BACTERIOLOGICAL EXAMINATION OF FECES.

This may be done by examining a particle of freshly passed feces by special culture media as outlined on page 216, to differentiate the colon-typhoid-dysentery group. Should the lesion be localized to the sigmoid or rectum better results will be obtained by passing a proctoscope and after swabbing off the ulcer with water remove a small piece of tissue by means of a sharp curette from the margin of the ulcer for culture. From this material either autogenous vaccine or specific serum may be prepared.

Examination for amœba is best made on fresh feces over a warm stage microscope. In amœbic dysentery the entamœba are in the active vegetative form when diarrhea is present and are passed in a resting encysted state between attacks when the patient acts as a carrier. It is best to examine for the vegetative forms within an hour although amœba will show motility six hours after the stool is passed. The feces *must* be kept warm as active amœba will lose their motility and not regain it if the stool is allowed to cool off. In examining for encysted forms this precaution is not necessary. A satisfactory heating arrangement may be made by placing an electric bulb under or near the microscope stage, draping a paper around the nosepiece and light and placing an ordinary clinical thermometer near the microscopic slide being examined. Endamœbæ will remain active in temperature anywhere between 90° and 100° F. and are best seen under a cover-glass with the high dry lens. When present they are usually found in shreds of mucus after a saline cathartic or in mucous masses removed through a proctoscope. The active motility and ability to ingest red blood cells distinguish the Endamœba histolytica which causes dysentery from other nonpathogenic forms which are sluggishly motile and ingest bacteria and other particles but do not ingest red blood cells. The presence of endamœba coli is evidence that the vegetable foods have been contaminated by feces and is highly suggestive that there may also be infection with Endamœba histolytica.[1]

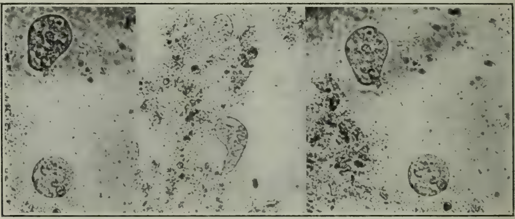

FIG. 91.—Unstained Amœba histolytica. × 500.

For examination of the encysted forms which occur in carriers and chronic cases, mix on a glass slide a small particle of feces or mucus with Lugol's solution. Cover with a cover-glass and examine under the high dry lens. The cysts of Amœba histolytica have only 4 nuclei in contrast to those of Amœba coli which have 8. For concentration methods consult special works on parasitic protozoa.

EXAMINATION OF INFANT'S STOOLS.

A personal inspection should be made whenever possible rather than reading the nurse's report. The stools should be investigated from a standpoint of number, size, color, consistency, odor, mucus, curds and blood.

Number.—The number varies. One a day is sufficient. More than four is considered pathological. When an infant whose habit it is to have one stool a day, suddenly begins to have three or four, diarrhea is imminent. When the stools are very numerous (10 to 20), the type of diarrhea is severe. This condition is caused by irritability of the colon which will not retain the fecal matter as it comes from the upper bowel.

Color.—An infant receiving barley, cereals or starches will have a brown stool. One fed on whole milk or other strong mixtures will have a pale yellow or green stool. A stool may be green yet firm, smooth and normal in every other respect. It may turn green on standing. In severe diarrheas the green color is only an indication that the food is passed rapidly through the bowels and that the bile has not had a chance to undergo complete change.

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[1] Faust: Endamœba Coli Index of E. Histolytica, Am. Jour. of Trop. Med., 1930, **10**, 137.

Consistency.—This depends on the food given. In the artificially fed it should be about that of stiff ointment and homogenous. It may be moistened with urine or if moist when passed may dry on the diaper. Usually the looser the stool the more severe is the diarrhea. Foamy stool is probably due to carbohydrate fermentation. Following a bout of diarrhea the stool is naturally hard.

Odor.—A sour odor is due to carbohydrate fermentation. This condition may be excited by a fatty indigestion. A foul putrefactive odor is typical of protein putrefaction.

Curds.—It is a disputed point whether or not curds appear in the normal stool. Protein curds are hard, tough and leathery. Fat curds, which are much commoner, are buttery in consistency. They can be made to disappear by boiling the milk. The soft curd is liable to be confused with flakes of mucus, but the former are generally white on the inside and green on the outside of the fecal masses.

Mucus.—This does not appear in normal stool and when present indicates an inflammatory condition of the bowel. The more mucus the more serious is the diarrhea. If it is well incorporated with the feces it comes from high up. If it is separated it comes from the lower bowel. It is a provision of nature for protecting the intestinal mucosa and therefore should not be removed by irrigation.

Blood.—When mixed with mucus and associated with frequent loose stools it is indicative of an infectious diarrhea, dysentery, ulcer, intussusception, tuberculosis or scurvy. A streak of blood on the outside of a hard stool or pure fresh blood associated with a normal stool is indicative of a fissure, ulcer, polypus or hemorrhoids of the rectum.

A Valuable Test for Differentiating the Severe Fermentative Diarrheas from the Infectious Types.—In the infective diarrheas or enteritis the inflammation of the bowel wall causes an exudate of polymorphs and lymphocytes into the bowel contents.

The Test.—Obtain a fresh specimen of stool by passing a catheter. Put a drop on a glass slide, add 2 drops of water, mix, brush the large particles aside and examine under the low power of the microscope. There will be hundreds of cells in each field in a case of enteritis and under 10 in the fermentative diarrheas.

CHAPTER XI.

CUTANEOUS TESTS.

EXAMINATION OF HAIRS FOR RINGWORM AND OTHER SPORE DISEASES.

Obtaining the Specimen.—Select an area partly denuded of hair. Dab this patch with absorbent cotton soaked in chloroform. This whitens most of the affected hairs. The hairs examined should be short broken ones and if they are not carefully selected the examination will be time wasted as the result will invariably be negative. With flat-tipped forceps extract five to ten hairs.

Simple Method.—Place the hairs in a teaspoon or watch-glass and cover with ether or chloroform. Move the hairs through the solution to dissolve off any fat from ointments which have been applied. Drain the ether off on a paper so any hairs may be recovered. Now half fill the spoon with 25 per cent sodium hydroxide and heat to boiling. Remove the hairs to a glass slide with a few drops of the solution. Add a cover-glass, press down on it and examine under the high dry lens. The hairs appear as yellowish shafts with a darkly pigmented central core. Very minute specks may be seen on the hair normally. Ringworm shows itself in two forms. (1) Spores which have the appearance of unstained red cells and are closely packed edge to edge over the surface of the hair. Sometimes they occur in rows. (2) Mycelial threads which have about the same width as the spores but they consist of long shafts sometimes having constrictions at intervals.

More Accurate Diagnostic Method Using a Stain.—Place 5 to 10 broken off hairs in a spoon or watch-glass.

1. Wash with ether. Drain on paper.

Modified Gram Stain.

2. Crystal violet two minutes. Drain on paper.
3. Gram's iodine two minutes. Drain on paper.
4. Aniline oil five to ten minutes. Drain on paper.
5. Xylol one minute. Drain on paper. Lay the hairs on a glass slide parallel to each other.
6. Add a large drop of Canada balsam and a cover-glass. Examine for spores and mycelial threads under the high dry lens.

The spores and mycelial threads are blue. The hairs may contain faint traces of blue and the nuclei only of the epithelial cells in the hair sheath retain the stain.

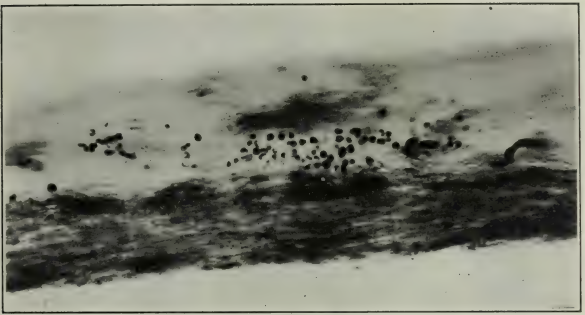

FIG. 92.—Stained spores of ringworm on the shaft of a hair under the high dry lens × 475. The upper border of the hair and the mycelial thread at the right which dips into the hair shaft, are out of focus.

Merits of the Unstained and the Stained Methods.—The unstained method is sufficient for one who is thoroughly acquainted with the microscopic appearance of spores and if many spores are present.

Ointment is usually applied to chronic lesions before a doctor sees it and a few fat droplets under epithelial débris may escape solution by the ether or hot sodium hydroxide. On microscopic examination they may have an appearance identical with a few scattered spores.

Sir Norman Walker states "If there is any doubt as to the nature of the case, the (liquor potassæ) method contains a good many fallacies which diminish its value." In a doubtful case one should always resort to the stained method. There is nothing one can confuse with Gram-positive spores or mycelial threads. If the slide is saved, it forms a permanent record.

EXAMINATION OF SKIN FOR FUNGUS DISEASE.

In collecting material for examination, it is better to use scissors and snip off parts of cuticle from the active margin of the diseased area, than to depend on the examination of scales scraped off by a penknife. Place the material to be examined in a watch-glass or spoon, add ether and after stirring for a few seconds, drain. As the cuticle is thick and opaque, it is necessary to render it translucent by adding a few drops of 25 per cent sodium hydroxide then heat to boiling for a few seconds. For direct examination, transfer the epithelium to a glass slide and thoroughly press out under a cover-glass. The spores and mycelial threads of any fungus present will, by their highly refractile properties stand out clearly. Later the cover-glass may be removed, the cells fixed to the slide by heat and stained by the modified Gram's method outlined for the staining of the spores on hairs. (See page 334.)

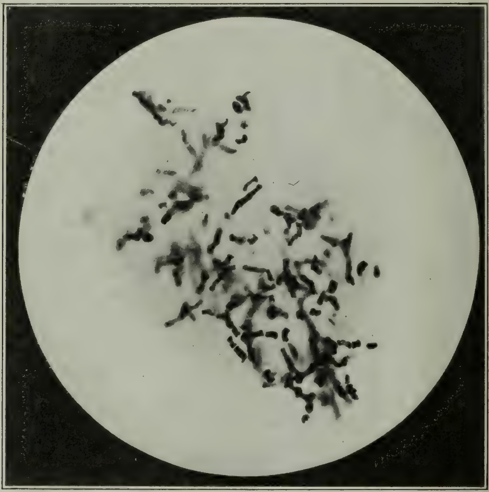

FIG. 93.—Stained specimen from a lesion of favus showing a mass of mycelial threads and a few spores. × 500.

Interpretation.—One should always examine the skin from an obstinate dermatitis for fungus, as the disease process is usually of long duration in fungus lesions. An examination should be repeated each time the patient sees the doctor until a diagnosis is made. By such efforts dermatologists are finding a specific fungus in lesions formerly labelled chronic eczema. Finding the spores and mycelial threads is more important than differentiating the type. After seeing spores and mycelial threads several times the eye becomes accustomed to their appearance and the variety may be settled by recourse to a text on skin diseases. At present as well as ringworm of the hair, the beard and the body, special fungus diseases occur in the groin, on the palms, on the soles and about the nails. Occasionally they invade the deep tissues, as in actinomycosis and blastomycosis.

ACTINOMYCOSIS.

Pus from an actinomycosis abscess contains small yellow bodies known as sulphur granules. If the pus be put in a test-tube of water the granules will sink in a few minutes and the pus cells which remain in the water may be drained off. The preparation for demonstrating the ray fungus is the same as that of ringworm. The granule has a characteristic appearance. Many club-shaped filaments converge to a homogeneous center. (See page 233.)

Interpretation.—Cattle are the commonest subjects of this disease where it usually takes the form of lumpy jaw. In man, actinomycosis locates around the mouth or throat. If treatment is not successful it will invade the vital organs or due to prolonged suppuration the patient dies from exhaustion.

CHAPTER XII.

REACTIONS OF IMMUNITY.

THE SCHICK TEST FOR SUSCEPTIBILITY TO DIPHTHERIA.

PRINCIPLE.—If persons susceptible to diphtheria have a minute amount of diphtheria toxin injected intradermally a red area develops. Those immune to diphtheria do not develop a redness at the site of inoculation. The natural antitoxin in the body neutralizes the toxin rendering it non-irritating. Susceptible individuals have no natural antitoxin.

Apparatus and Reagents.—Concentrated toxin in a capillary tube and a 5-cc. vial of saline in which to dilute the toxin. Diphtheria bacillus protein solution or toxoid as a control test. N.B.—The package containing the toxin should not be outdated. It should be kept in a refrigerator until used and diluted immediately before injection.

Two 1-cc. tuberculin syringes and new 26- or 27-gauge steel needle which fits tightly to the tuberculin syringe.

Alcohol and absorbent.

Diluting the Toxin.—Break off both ends of the capillary tube without losing any of the contents. Insert one end of the capillary tube into the rubber bulb for expelling it. Hold the bulb between the thumb and middle fingers. Place the index finger over the top of the bulb and expel the contents into the vial of saline. Draw up and expel saline from the capillary tube several times to rinse out all the toxin. Cork the vial and invert several times to mix rather than shaking it up which weakens the toxin. While the concentrated toxin in the capillary tube keeps well it rapidly loses strength when it is diluted and should be used within twenty-four hours.

The Control Material.—Some manufacturers supply toxoid as a control test which not only indicates the sensitiveness to diphtheria bacillus protein but is a guide to the dosage. Where this is not supplied the toxin may be destroyed without altering the diphtheria bacillus protein by placing one-half the diluted toxin in a test-tube and immersing it in a water-bath at 75° C. for ten minutes.

The Test.—Inject exactly 0.1 cc. between the layers of skin on the flexor surface of the forearm. This is best accomplished by pinching

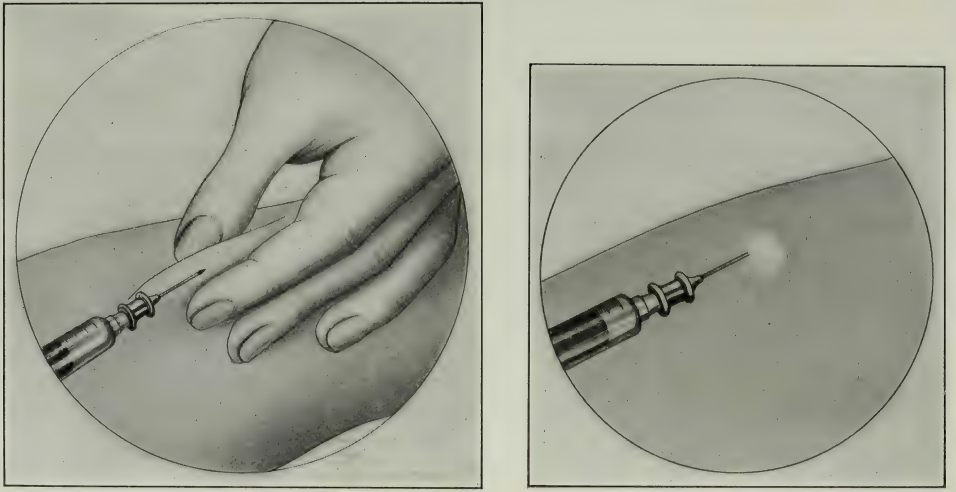

Fig. 94 Fig. 95

Figs. 94 and 95.—Technique for the intradermal test. Fig. 94. Introducing the needle with its opening up. Fig. 95. The fluid introduced will make a white wheal if it does not escape on the surface or subcutaneously. The correct dose intradermally is a necessity in the Schick test.

up a fold of skin between the thumb and index finger of the left hand and with the syringe in right hand so that the lumen is up, insert the point into the fold of skin until the lumen disappears. Then inject exactly 0.1 cc. If properly done the skin raises in a small white wheal except where held down at the hair follicles. Sometimes injection does not produce this white wheal and will not give a satisfac-

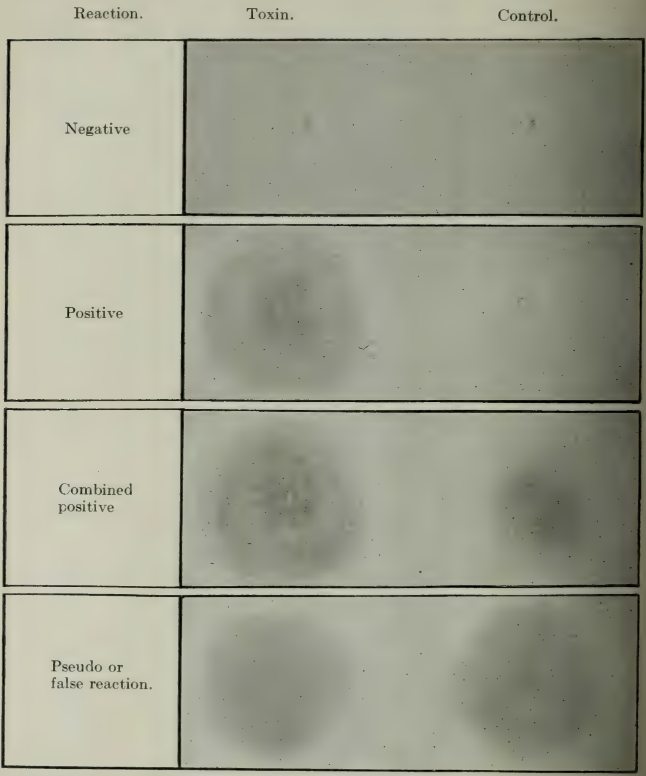
Reaction.
Toxin.
Control.
Negative
Positive
Combined positive
Pseudo or false reaction.

Fig. 96.—Schick test reactions (four days after injections). The pseudo reactions, which occur in the control, fade after the third day while an increase in size takes place in the true positives leaving a brownish scaling pigmentation which gradually fades in a few weeks. When only one reading can be made it should be done between the fourth and the seventh day.

tory test. If the fluid goes too deep or if any is lost on the surface repeat the test in another place. Inject the control test material 2 inches below the site of toxin injection on the forearm.

If toxoid be used as a control test and it shows a reaction while the toxin does not, the patient is immune. When both show a reaction it is difficult to say whether it is a combined (positive) or a pseudo (negative) reaction. If the toxin reaction reaches its height on the third or fourth day and a brownish pigmentation persists for several weeks it is likely positive and the patient should be immunized starting with one-half the first dose.

The reaction in the control test occurs and disappears sooner than the reaction to the diphtheria toxin. Children under eight years of age rarely show a reaction in the control. Adults show more pseudo (negative) reactions than positives.

The negative reaction or the pseudo reaction may be taken as a sign of immunity to diphtheria provided the proper dose of test material has been placed between the layers of the skin. Sources of error are, the use of material after the expiration date on the package or if it has not been kept in a refrigerator; loss of strength from shaking diluted fluid toxin or not using it immediately after dilution; injecting the 0.1 cc. too deeply or losing part of it due to the needle fitting on the syringe poorly. A very large percentage of negative reactions especially when occurring among children should make one suspicious of defective material or technique.

Interpretation.—The Schick test is very reliable. Only individuals showing a positive reaction will take diphtheria. Less than 15 per cent of newly-born infants will give positive Schick reactions. This corresponds to the number of mothers giving positive reactions and liable to diphtheria. The other 85 per cent of infants are immune, due to immunity derived from the mother, which lasts six to nine months. Ninety per cent of children at one year of age give a positive reaction and at five years 60 per cent give a positive reaction. This high percentage of susceptibility explains the high mortality at the age of one to five years. Over 60 per cent of all deaths occur in children under five years and 80 per cent in children under ten years of age. Fifty per cent of nurses coming from rural sections react positively to the Schick test because they are not exposed to slight immunizing infections. All children from six months to six years of age should receive active immunization, the earlier ages preferably, but under six months immunity can be but poorly developed.

Dosage.—In treating adults severe reactions are liable to occur and when the tenth cubic centimeter used as a control in the Schick test gives a reaction over 1 inch in diameter on the second day, it is best to inject only one-half of the first dose and if the reaction to this half dose be marked to increase the dosage by cautious steps. Those who do not show a reaction to the control toxoid in the Schick test may receive 3 subcutaneous doses, each at intervals of three weeks. A three-week interval develops a better immunity than does a one-week interval. A Schick test applied two months after the last injection will detect any who are not successfully immunized. This however is less than 5 per cent and such individuals should receive further treatment. Once the immunity is acquired it lasts for many years, perhaps a lifetime. In an outbreak of diphtheria at a school or children's home the best procedure is:

1. The examination of all throats for membrane, taking a swab for culture at the same time.
2. Immediate administration of 5000 to 50,000 units of antitoxin to all cases showing any sign of membrane or having difficulty in breathing. One should not wait for a bacteriological report to decide whether or not a membranous exudate is diphtheritic.
3. Apply the Schick test to those not affected, then immunize all Schick-positive cases by a series of toxoid injections.

Where there has been very close contact it is best to give a prophylactic dose of diphtheria antitoxin, performing the Schick test and immunizing at a later date if necessary. The aim should be to do a Schick test on all children and immunize the susceptible ones before there is any alarm from contact with actual cases of diphtheria.

The Carrier State.—A large number of patients lose their bacilli a few days after the membrane disappears and in the remainder the bacilli gradually die out. Carriers of long-standing usually have infected tonsils, adenoids or sinuses and surgical treatment is of the greatest value. Two negative swabs on successive days are usually required for release from quarantine. This will detect only the profuse carrier who is as infectious as an acute case of diphtheria. A scanty carrier may periodically develop into a profuse carrier, giving to a negative report a false sense of security. Some institutions have abandoned the negative requirement and discharge the patient when the throat has a normal appearance and if there is no nasal discharge. Extensive examination has shown that about 3 per cent of school children harbor in their nasopharynx, organisms that have the morphology of Bacillus diphtheria but virulence tests show that only 0.3 per cent harbor virulent organisms. Sorting out the susceptible children and making them immune is a more successful means of prevention than trying to locate chronic carriers and isolate them.

THE DICK TEST FOR SUSCEPTIBILITY TO SCARLET FEVER.

What has been said about the Schick test for immunity to diphtheria might be repeated about the Dick test with the following exceptions:

1. The Dick test is not quite such a reliable indicator of susceptibility to scarlet fever as the Schick test is to diphtheria.

2. The Dick reaction occurs in twenty-four hours and a control test is not necessary.

3. The positive Dick reactions and frequency of the disease show that susceptibility occurs at an earlier age than in diphtheria. From birth to six months of age 30 per cent show positive reactions. From one to two years of age 70 per cent, at ten years 50 per cent and after twenty years 25 per cent.

The Dick test will probably be abandoned in children under ten years of age, as a very high percentage show a positive reaction. It is useful to determine susceptibility in nurses or in boarding schools where only 20 to 25 per cent are susceptible.

The Dick Test.—Inject 0.1 cc. of diluted scarlet fever streptococcus toxin intradermally using the technique advised in the Schick test. Formerly a control was employed. It consisted in injecting 0.1 cc. of the scarlet fever diluted toxin after it had been immersed in a water-bath for an hour, the toxin being more resistant than that of diphtheria. Recently this has been dispensed with.

Reactions.—The reaction occurs in twenty-four hours and after that soon disappears. It consists in a red blush $\frac{1}{2}$ inch or more in diameter about the site of toxin injection.

Interpretation.—A positive reaction signifies that the patient has no natural antitoxin and is therefore susceptible to scarlet fever. Those showing a negative reaction are immune to scarlet fever.

The test has other uses however. The reaction is positive in those suffering from scarlet fever during the first three days of the disease. After that it becomes negative until at convalescence it is usually negative. If a patient shows a negative reaction during the first three days of a fever that fever is usually not scarlet. Also if after a week or so of fever the Dick test is positive it weighs against a diagnosis of scarlet fever.

Recent experiences with the Dick test by Japanese workers in Manchuria are instructive. They agree that the hemolytic streptococcus is the sole etiological agent in scarlet fever.[1] They point out that from 16 to 33 per cent of children who had scarlet fever are

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[1] Toyoda, *et al.:* Lancet, 1930, **217**, 73.

Dick positive to some commercial preparations of toxin whereas less than 0.1 per cent had a second attack of scarlet fever; that 13 per cent of adults usually show a positive Dick reaction although there is a very low incidence of scarlet fever among them; that 10 to 30 per cent of those convalescing from scarlet fever show positive reactions; that serum from a person showing a positive Dick reaction may sometimes produce a positive Schultz-Charlton reaction. These discrepancies are overcome by preparation of a toxin free from nucleoprotein of the streptococcus. This nucleoprotein, being somewhat common to all streptococci, is the cause of non-specific reactions.

ACTIVE IMMUNIZATION FOR THE PREVENTION OF SCARLET FEVER.

Recently the originators of the Dick test[1] have shown that active immunity can be produced by injections of the toxin. The large number of injections necessary is a drawback to the practical application of this procedure. They advise the use of sterile toxin, starting with 500 units and increasing by 5 injections, one given each week, the last injection containing 100,000 units. After so treating over 10,000 susceptible individuals they find that 95 per cent are completely immunized as shown by skin tests and actual exposure to the disease. The others are partially immunized. Over 90 per cent retain full immunity for three years, the remainder requiring a second course of immunization. This work is too recent to determine the full length of time the immunity lasts but it is quite likely that it will bridge across the years of susceptibility to scarlet fever in childhood. Partial replacement of the toxin by the specific protein of the scarlet fever streptococcus holds promise of reducing the number of injections and giving an immunity of high degree and long duration.

SCHULTZ-CHARLTON REACTION. RASH EXTINCTION TEST FOR SCARLET FEVER.

Inject 0.5 cc. of antiscarlet fever serum into the skin in an area showing a bright rash. After six hours an area of skin ½ to a few inches in diameter has a normal color. The margin is usually sharply defined. There is a small red central area from trauma of the injection.

The serum from a convalescent scarlet fever patient and most adults who normally have an immunity to scarlet fever will blanch the rash in the same manner as antiscarlet fever serum.

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[1] Dick and Dick: The Control of Scarlet Fever, Am. Jour. Dis. of Child., 1929, **38**, 900.

SERUM TREATMENT OF SCARLET FEVER.

The beneficial action of scarlet fever antitoxin is rapid, effective and it also prevents the development of complications. Due to improper standardization, some of the commercial serums are inert, although they are labelled to neutralize 30,000 skin doses per cubic centimeter.[1] The disease is frequently very mild and in such cases more discomfort is experienced by the serum reaction than there would be benefit accrue by giving serum. In all severe cases of scarlet fever, serum should be administered. It should be given at the earliest possible moment because in the late stages the antiserum can only partially neutralize the toxin and so the result is not satisfactory. Park advises against the use of serum to immediate contacts. He prefers to do a Dick test and to give active immunization to those showing positive reactions rather than giving the serum, which produces immunity only for two weeks and which may produce urticaria.

Mode of Scarlet Fever Contagion.—The scarlet fever streptococcus is hemolytic. The tonsil is the principal focus and none are found in the cutaneous scales.[2] The streptococcus is also present on the beds, walls, and in the air of the room. The presence of hemolytic streptococci in the throats of patients is not indicative of scarlet fever as they also occur in common angina. Their absence is good evidence against the infection, however. By moving patients to a convalescent ward when their infection is less profuse it is possible to render them free from hemolytic streptococci in three to five weeks. They are discharged when 3 cultures from throat swabs are negative. A bacteriological examination is a better criterion for discharge than routine dismissal after six weeks and after the disappearance of scales.

VACCINATION AGAINST SMALLPOX.

Supply of Vaccine.—To insure potency vaccine should not be used after its expiration date and during that time it should be stored in a refrigerator. Vaccine consists of a very resistant virus stored in an antiseptic solution. Heat enhances the action of the antiseptic and weakens the virus so that if warmth is applied for over a month, the virus will become completely inert. Storage in the vest pocket or at room temperature for a day, in a cellar for a week or in a refrigerator at 4° C. for a month will not appreciably weaken a vaccine.

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[1] Wadsworth: Scarlet Fever, Am. Jour. Pub. Health, 1929, **19**, 1287.

[2] Friedman: Lancet, 1928, ii, 211.

Vaccine should not be transported by ordinary mail where it is liable to be subjected to considerable heat. A refrigerator box packed with ice is the proper method. A lack of potency should be suspected in any vaccine that does not give over 90 per cent of typical vaccinia reactions in those who have not already been successfully vaccinated.

Method.—The multiple pressure method is most suitable because it insures a very high percentage of takes. The very slight trauma makes it a painless procedure and as there is no break in the epidermis there is little chance of secondary infection.

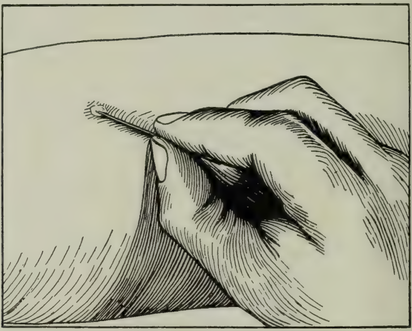


FIG. 97.—Multiple pressure method of vaccination.

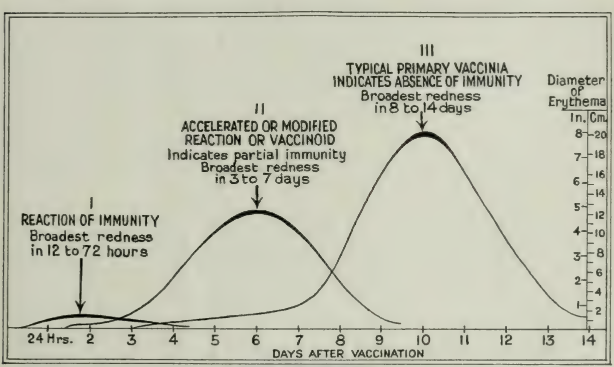
The best site for vaccination is on the upper arm at the insertion of the deltoid because of the minimum of movement and of blood-vessels. Cleanse the area with alcohol or acetone and allow it to dry. Cleanse the capillary tube containing the vaccine by wiping it with a sponge dipped in alcohol. Break off the ends of the tube and by means of the rubber bulb, expel a drop of vaccine on the arm. Inoculate by pressing the side of the point of a sterile needle into the drop so that the point will just make an indentation on the skin. Make 20 to 30 such impresses by rapidly striking the skin over an area of $\frac{1}{8}$ inch with the side of the needle point. Wipe off the excess vaccine with sterile gauze. Little or no skin lesion is visible. The virus is deposited between the epidermal cells, where it regenerates. Do not apply a vaccination shield. For mild reactions several layers of gauze stitched to the garment which covers the lesion is best. Very severe cases may require a light porous surgical dressing for a few days.

Some physicians rub the vaccine into two tiny scratches about

$\frac{1}{16}$ inch long and about $1\frac{1}{2}$ inches apart. This method gives a high percentage of takes. Vaccination on the leg should be discouraged because the patient is compelled to go to bed while the vaccinia is active. The scar on the arm is slight when modern methods are used but if the patient insists on vaccination on the leg make the inoculation on the outside of the thigh.

Reaction.—Three types of reaction occur:

1. Immediate reaction twelve to seventy-two hours after vaccination. A red area $\frac{1}{2}$ inch in diameter which soon fades indicates the reaction occurring in those who are immunized by previous successful vaccination or by an attack of smallpox. Later reactions indicate lack of immunity.



After J.P. LEACH M.D.
U.S. Public Health Service

FIG. 98.—Types of reaction in smallpox vaccination.

2. Reaction three to seven days after inoculation consisting of a red area with small vesicles in the center indicates the reaction of partial immunity or vaccinoid occurring in those who have been successfully vaccinated five to ten or more years previously.

3. Reaction of typical vaccinia indicating absence of immunity occurs eight to fourteen days after the inoculation and has an area of redness 6 to 8 inches in diameter with vesicles containing a turbid grayish fluid. This produces an immunity which lasts five to ten years after which it slowly diminishes.

Facts About Vaccination and Smallpox.—A person in whom no vaccination has taken is not naturally immune from smallpox. Failure to "take" was likely due to a weak vaccine virus. I have seen a typical vaccinia develop in a medical student after vaccination although he had previously been fourteen times unsuccessfully vaccinated.

The mild reaction of partial immunity occurs almost unfailingly in those who have already had a successful vaccination.

Among the unvaccinated less than 7 per cent escape smallpox in a severe epidemic. In mild epidemics a larger percentage is spared. The fatality among the unvaccinated is more than 50 times the fatality among those vaccinated at *any* time in their life.

Children should be first vaccinated at two to six months, again at five to seven and at fifteen years of age. Vaccination four days after exposure to a case of smallpox may prevent the disease. Persons likely to exposure to the disease would be revaccinated every ten years. Vaccination affords the most perfect immunity we have in medicine to a communicable and fatal disease. Its value is emphasized by the fact that no remedy is of any avail once smallpox develops.

CONVALESCENT SERUM FOR MEASLES AND POLIOMYELITIS.

The Donors.—The blood of convalescent patients who have been free of fever from a week to a month is best. In poliomyelitis the serum is of value up to two years after the patient has recovered and in measles there is no very definite decrease during the first two months. After these dates the immunity decreases gradually and then remains at a low constant indefinitely which makes a recurrence of the disease very unusual. The donor should be free from syphilis or tuberculosis.

Where there are no facilities for obtaining convalescent serum the injection of whole blood may be used. The fact that a small but permanent immunity remains after an attack of measles or poliomyelitis makes the blood of those who have ever had the disease of considerable value. Most parents have had measles and they will willingly give blood for their children. Dr. William H. Park has found this procedure of value. The blood is removed with a 20-cc. syringe and injected immediately into the gluteus muscle of the patient requiring the protection.

Obtaining the Serum.—Under aseptic precautions collect the blood in large test-tubes using an 18- to 20-gauge needle and place in a cold room overnight. Loosen the clot from the sides of the tube and centrifuge for one-half hour. Pipet the serum off under sterile precautions and if for poliomyelitis make sure that little or no hemoglobin is present as this may produce a severe reaction if injected intraspinally. Test 0.5 cc. of the serum for sterility and if sterile, as a preservative add 0.8 cc. of 25 per cent solution of cresol to 100 cc. of serum. The serum does not lose its power for a year after the date of bleeding.

Administration.—Convalescent serum and blood is of value in any disease in which one attack confers immunity. The amount of whole blood should be double that of serum and the amount should be doubled or trebled when the donor's attack of the disease has not been recent.

In measles 10 cc. of convalescent serum injected subcutaneously or intramuscularly will protect an individual for about a month. Even five or six days after exposure to the disease the serum will prevent the development of measles. If serum be injected after the sixth and before the ninth day of incubation a mild attack of measles will develop and the patient will acquire considerable immunity. The fifth or sixth day after exposure is best.

Convalescent serum is of value in the pre-paralytic stage in poliomyelitis. One dose may be given intravenously when the patient is first seen. This should be followed by 2 intraspinal injections each of 15 to 20 cc. at twenty-four-hour intervals. Smaller doses should be given to small children.

TYPHOID VACCINATION.

For the prevention of typhoid fever inoculations with typhoid vaccine have great value. This has been thoroughly proven during the World War. The typhoid vaccine is put up with a 1000 million typhoid and 750 million each of Para A and B to each cubic centimeter of vaccine. Three doses are given at weekly intervals. The first dose is 0.25 cc., the second 0.5 cc. and the third 1 cc. The Widal reaction will show partially positive some weeks after the last injection indicating a definite immunity which lasts three to five years after which time it diminishes.

Typhoid Vaccine as a Foreign Protein.

Action and Indications.—Here the vaccine is diluted because the reaction from undiluted vaccine is too severe and liable to prove fatal if given intravenously.

This mode of treatment is of value if there is a subacute or chronic localized inflammatory lesion such as iritis, arthritis, fibrositis or orchitis, but the general health good. It should not be administered to patients if they are elderly, if they have a feeble constitution or an impaired myocardium. In the usual reaction, which occurs in less than an hour, the patient develops severe rigors, frontal headache, abdominal pain and perhaps vomiting. There is also intense pain in the back, in the joints, in the long bones and in the diseased focus. The temperature after a short dip below normal mounts to over 100° F. and the pulse increases. After fifteen to thirty minutes of this, a profuse perspiration breaks out and the pain and rigors diminish, leaving the patient relaxed and frequently feeling better. When such a reaction occurs there is a definite increase in the general resistance as shown by the elevation of temperature and the increase in the leucocytes to two or three times their normal. This together with the increased exudation of serous lymph into the diseased area, brings about a more rapid and effective cure than the natural but mild stimulation of the bodily resistance produced by the lesion itself.

Method of Administration.—In a sterilized 1-cc. tuberculin syringe take exactly 0.1 cc. of a well-shaken sample of typhoid vaccine. Add 0.9 cc. of sterile water and a bubble of air. Tilt the syringe several times so as to thoroughly mix the vaccine and water. Expel the air bubble and part of the diluted vaccine.

One-twentieth cubic centimeter of this vaccine so diluted contains 12½ million typhoid bacilli and is the best dose with which to begin treatment. It is one-half the amount usually given in the inital dose and if no reaction occurs, 0.1 cc. which contains 25 million bacteria, may be administered the following day. Inject slowly into any prominent vein at the bend of the elbow, using a small hypodermic needle on the tuberculin syringe and measuring the dose accurately.

If a reaction is delayed more than two hours it is a sign that the patient's resistance mechanism does not respond to the stimulus of the bacterial protein in the blood stream. In such cases further injections offer no therapeutic hope and may even be definitely harmful. Those who benefit by intravenous foreign injection develop the reactions promptly in less than an hour and injections should be continued, increasing the dose to 50, 75, and 100 million at intervals of from five to seven days. If the reactions are severe give smaller doses. If benefit occurs it will be obtained by 5 injections. Should the original symptoms recur months or years later the course of injections may be repeated.

TUBERCULIN REACTIONS.

There are three methods of applying the tuberculin test, the cutaneous method of Pirquet, the intracutaneous injection and the subcutaneous injection.

The cutaneous method of Pirquet is the most easily applied and usually shows satisfactory reactions. The intracutaneous method is more sensitive but there is a considerable chance of error in obtaining the correct dose by dilution and injecting it properly between the layers of the skin. As there is little or none of the tuberculin absorbed into the circulation there is practically no danger of a constitutional reaction.

The subcutaneous injection is not without danger of reaction which is harmful if there is an active pulmonary tuberculosis. A positive result is a general reaction in the form of an elevation of temperature and a focal reaction at the site of any tuberculous lesion due to an increase in the exudate from it. This accentuates the local symptoms.

All tuberculin treatment is given subcutaneously and should start with very minute doses.

Material.—Koch's old tuberculin,[1] 1-cc. vial. This keeps its strength indefinitely at room temperature and the high percentage of glycerin in it prevents bacterial contamination.

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[1] *Preparation of Dilution.*—Dilutions, especially if very weak, deteriorate in ten to fourteen days. When only small amounts are required for the test dilutions are most economically prepared in a sterile pipet for red cell count. Draw concentrated old tuberculin up to mark "1" and sterile saline or water to "101." After shaking the pipet to mix the contents in the bulb and blowing out the undiluted water in the stem, the solution in the bulb will be 1 in 100 dilution. If this 1 in 100 be again diluted 1 in 100 the resulting solution is 1 in 10,000. If 1 part of a 1 in 10,000 solution be diluted 100 times the resulting solution is 1 in 1,000,000.

Cutaneous Test (*Pirquet*).—Sterilize the forearm with alcohol. Make three scratches on the arm one above the other. Apply, using a sterile needle or applicator, a drop of undiluted old tuberculin to the top scratch and then rub what is left on the same applicator into the lower scratch leaving the center one as a control. Examine in twenty-four to forty-eight hours. More inflammatory redness at the point of the tuberculin inunction than in the control scratch indicates a positive reaction.

Interpretation.—Tuberculin produces reaction only in those who harbor tuberculous infection. Most individuals acquire a mild tuberculous infection which heals up early in life and these show a reaction in the same manner as when clinically active tuberculosis is present. It tells the presence or absence of tuberculosis but does not indicate its activity.

The incidence of infection in city populations is as follows:

At 1 year 5 per cent positive reactions.
At 2 years 10 per cent positive reactions.
At 3 years 25 per cent positive reactions.
At 6 years 50 per cent positive reactions.
At 14 years 85 per cent positive reactions.

The tuberculin test finds its greatest use in the diagnosis of infection of children under two years of age.

Because of the prolonged course of tuberculosis one may assume that *a positive reaction in children under two or three years of age means active infection* whereas in adults the lesions have had time to heal. A negative reaction excludes the presence of tuberculous disease in all but the moribund.

Intracutaneous Tuberculin Test.—This method is more sensitive and accurate than the scratch method of Pirquet. It should be used when the scratch method gives a negative result and there is still grave suspicion of a tuberculous lesion. The interpretation is the same as in the Pirquet test.

Apparatus and Reagents.—A 1-cc. tuberculin syringe with a sharp, well-fitting 27-gauge needle. Freshly diluted tuberculin, 1 in 10,000.

Method.—Cleanse the skin with an alcohol wipe and pinch a fold of it up between the thumb and index finger of the left hand. With the needle parallel to the skin surface and its lumen up, insert it into the superficial layer of the firmly held fold until the lumen disappears. Inject exactly 0.1 cc. between the layers of the skin. No fluid should escape on the surface and if the injection is too deep no skin change is seen. A correct intradermal injection produces a blanched elevated wheal in the skin. Use a 1 in 10,000 dilution and if the reaction be negative repeat using 1 in 1,000.

Subcutaneous Tuberculin Test.—Here the focal phenomenon is of value in determining whether a lesion in an adult is tuberculous. If the symptoms from a lesion are increased by an injection of tuberculin the lesion is likely tuberculous.

Apparatus and Reagents.—One cubic centimeter tuberculin syringe; 1 in 1,000 fresh tuberculin.

Method.—Keep the patient in bed for a day or two before the test and record the temperature and pulse every twenty-four hours. Only apply the test if the temperature is normal and the lungs show little or no tuberculous infection on *x*-ray and physical examination.

Inject subcutaneously 0.1 mgm. (0.1 cc. of a 1 in 1000 dilution) of old tuberculin and record the temperature and pulse every two hours for two days. If no reaction occurs repeat the injection using 0.5 mgm. (0.5 cc. of 1 in 1000 dilution). Should no reaction occur to this, 1 mgm. (1 cc. of 1 in 1000 dilution) may tried. A negative reaction to 1 mgm. is a good guarantee that no tuberculous infection is present.

A rise of 1° of temperature is positive. If positive one or all of the following reactions may occur.

The Local.—An inflammatory reaction around the site of injection.

The Focal.—A reaction occurring in tuberculous areas six to twelve hours after the injection and giving rise to increased exudation, pain and tenderness. Should any *x*-ray shadows in the lungs be tuberculous, they become more hazy, râles develop and the sputum becomes more copious.

The General.—Manifested by a rise in temperature above 99° F. with headache, chilliness and muscular pains, usually six hours after the injection.

Selection of Cases for Tuberculin Treatment (Dr. E. R. Long).—This mode of treatment should be undertaken with much caution. Pulmonary tuberculosis which is a diffuse process is more likely to be spread rather than cured by tuberculin. Favorable cases for tuberculin treatment are localized tuberculosis such as occurs in bones and joints, kidney, lymph gland, skin and eyes, especially if the lesions are sluggish and slowly progressive. It is a stimulating therapy. Doses which are too large will produce an excess of exudation and spread the disease. No patient with a fever over 100 should receive tuberculin injections.

Therapeutic Dosage.—To afebrile patients with a sluggish localized tuberculous focus, 0.1 cc. of a 1 in 1,000,000 dilution is a safe initial dose. As the tolerance develops increase the dose. Repeat at weekly intervals to make it one and a half times the dose previously given. Do not increase when any slight general reaction has occurred. The best results come from doses just large enough to cause a mild focal reaction without general reaction. Continue the injections as long as the patient improves.

CUTANEOUS REACTIONS TO PROTEINS.

PRINCIPLE.—If a protein substance causes edema of nasal or bronchial mucosa, it will usually show an urticarial wheal when rubbed into a scratch on the arm.

Reagents and Apparatus.—Powdered proteins and pollens in vials, sewing needle, toothpicks, alcohol and gauze wipes.

It is better to do 30 tests using individual proteins than to use group proteins in which 150 substances may be tested by 30 scratches. Dried pollens and proteins are supplied by H. K. Mulford Company, the Arlington Chemical Company and others, in 50 mgm. vials which is sufficient for 20 tests and they do not deteriorate in a year.

Pollens.—June grass, timothy, ragweed low and tall, with sagebrush added for the Western provinces.

Animal Emanations.—Horse, dog, cat and rabbit hair, chicken, duck and goose feathers.

Vegetable Dust.—Orris root, floor sweepings from the patient's house.[1]

Foods.—Whole egg, cows' milk, wheat, oats, potato, cabbage, lettuce, beans, tomato, orange, peanut, beef, mutton, chicken, salmon, lobster, cocoa.

In testing out 200 cases with an extensive list of 275 pollens and proteins, 90 per cent of the reactions occur in the above list of 30 proteins. If the following 47 be added to the above 30, 99 per cent of the genuine reactions obtained with the list of 275 pollens and proteins will be detected.

Pollens.—These will vary according to the locality but the most frequent reactions will occur with the following:

Spring.—Willow, maple, pollen from nearby orchard trees.

Summer.—Red top, orchard grass, rye grass, dandelion.

Fall.—Marsh elder, cocklebur and wormwood.

Dusts.—Cow hair, muskrat, fox, cotton seed, flax, sheep's wool, glue, tobacco.

Foods.—Subproteins of egg, ovomucoid, ovovitelline, egg-white, egg-yolk. Subproteins of cows' milk, casein, lactalbumin, peptone and proteose. Subproteins of wheat, leucosin, gliadin, glutenin, globulin and proteose. Beet, cauliflower, celery, rice, pork, veal, whitefish, apple, banana, mayonnaise, mustard, paprika, pear, walnut.

Keep the protein vials grouped in envelopes labelled "vegetables and cereals," "meat and fish," "milk and eggs," "hairs and feathers," and "pollens."

The Test.—Wash the arm with alcohol and after it dries make 2 or 3 rows of 10 to 15 small scratches, $\frac{1}{8}$ inch long and 1 inch apart, by strokes on the skin with the point of a needle held almost flat. By means of a medicine dropper place a small drop of tenth-normal solution of sodium hydroxide or approximately 1 per cent sodium chloride solution beside each scratch. Inoculate the scratches, beginning with the top one in the left hand row, by taking on the end of a toothpick a small amount of powdered protein from a vial, placing it on the scratch and rubbing in the drop of sodium hydroxide beside it to dissolve the protein. Use a fresh toothpick each time so as not to mix the proteins. Place in a row the vials from which the proteins have been used in the test, separating each group such as hairs, pollens, etc., by means of a toothpick and mark off the corresponding group of inoculations with an ink stroke. Should a reaction occur in one, say the fifth, in the series of scratches, the fifth vial in the corresponding series may be located and its contents recorded from the label.

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[1] When a patient complains that house dust will provoke hay fever or asthma, obtain from his house a mass of floor sweepings or, better, the contents of a vacuum cleaner. Extract it with an equal volume of tenth-normal sodium hydroxide. After standing overnight to effect solution, strain off the fluid and centrifuge it for one-half hour at high speed. The top of the solution will be sufficiently bacteria-free to use in the scratch test. Should a reaction occur, and it is impossible to keep the patient away from house dust, manufacturers of test proteins will prepare suitable sterile extracts for treatment from a sample of the dust.

Interpretation.—To be considered as positive it is necessary that a scratch have a larger wheal and surrounding blush than the negative ones which serve as controls. The reactions with an irregular margin denote more hypersensitiveness than one with a smooth margin. As reaction to foods there is frequently only a slight wheal and a large red blush. Slight reactions rarely have any clinical significance but if the substance be a food it is worth a trial of omitting it from the diet for four or five days.

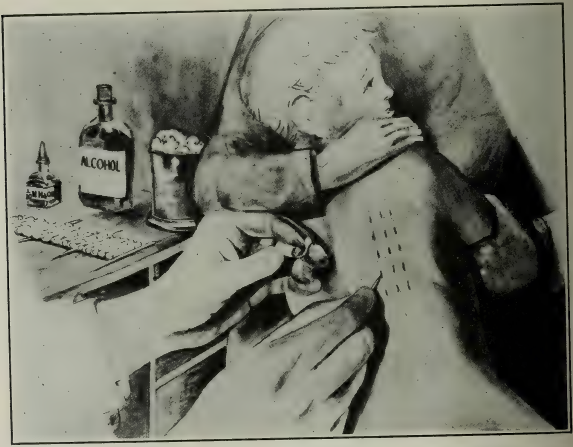

Fig. 99.—Method of obtaining cutaneous protein reactions in a child.

Reaction Diameters.

	Wheal.	Blush.
Slight	3 mm. (1/8 inch)	1 cm. (2/5 inch)
Definite	1 cm. (2/5 inch)	2 cm. (4/5 inch)
Marked	2 cm. (4/5 inch)	3 cm. (1 1/5 inch)

The larger the wheal the more definitely is the cause established. About 60 per cent of hay fever and asthmatic patients from three to thirty-five years of age show protein reactions. Cutaneous protein tests are useful in children and young adults suffering from hay fever and spasmodic asthma. Infants show most reactions to foods such as egg, while young adults show reactions most frequently to inhaled products such as animal hairs and feathers. Very rarely do patients past middle life suffering from a continuous type of asthma show cutaneous reactions to proteins and it is better to rely on autogenous vaccines from the sputum. There are very few adults suffering from urticaria or eczema who show positive cutaneous reactions to proteins.

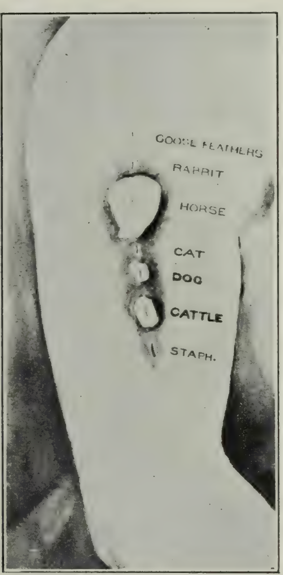

Fig. 100.—Cutaneous protein reactions consisting of a raised central wheal surrounded by a hyperemic blush. The reaction is marked to horse hair, moderate to cattle hair and slight to dog hair.

DESENSITIZATION.

Where the patient cannot avoid the offending substance which may be a pollen or horsehair it is advisable to desensitize him by increasing hypodermic injections. By this means the tolerance may be increased. The usual dose in commencing treatment is 0.1 cc. of 1 in 10,000 protein solution. It is better to make 3 scratches and place a drop of 1 in 10,000 in one, 1 in 1000 in the second, and 1 in 100 in the third. After ten minutes a reaction will occur with the strong solution. As first dose give 0.1 cc. of the strongest solution that does not produce a wheal not more than $\frac{1}{8}$ inch in diameter. The suggested outline is a satisfactory one although it is sometimes necessary to start with 0.1 cc. of 1 in 100,000.

Keep protein extract in a cool place. Give three hypodermic injections weekly of 1 in 10,000 according to the following scale:

0.1 cc. or 1 minim.
0.15 cc. or 2 minims.
0.2 cc. or 3 minims.
0.3 cc. or 5 minims.
0.5 cc. or 7 minims.
0.7 cc. or 11 minims.
1.0 cc. or 15 minims.

Then give two injections weekly of 1 in 1000 as follows:

0.1 cc. or 1 minim.
0.15 cc. or 2 minims.
0.2 cc. or 3 minims.
0.3 cc. or 5 minims.
0.5 cc. or 7 minims.
0.7 cc. or 11 minims.
1.0 cc. or 15 minims.

Follow with two injections weekly of 1 in 100 as follows:

0.1 cc. or 1 minim.
0.15 cc. or 2 minims.
0.2 cc. or 3 minims.
0.3 cc. or 5 minims.
0.4 cc. or 6 minims.
0.5 cc. or 7 minims.
0.6 cc. or 9 minims.
0.7 cc. or 11 minims.
0.8 cc. or 12 minims.
0.9 cc. or 13 minims.
1.0 cc. or 15 minims.

For Hay Fever.—Too many injections before each hay fever season is objectionable. Continuing the maximum dose every three weeks is under trial as a substitute for repeated pre-seasonal treatment. Severe reactions frequently occur so that it may be necessary to give only half the maximum dose.

Doses can be most accurately measured by a 1-cc. tuberculin syringe. The reactions of individuals vary. When they are very sensitive the dose must be diminished. If there is any exacerbation of symptoms or if the local reaction is more than 3 inches in diameter, repeat the dose last given and work up again at one-half the rate on the suggested scale. Any immediate unpleasant symptoms may be controlled by 0.5 cc. of 1 in 1000 adrenalin. The essentials in increasing the tolerance are, treatment beginning with a small dose which is gradually increased over a long period of time. The process is very tedious, desensitization never complete and when the treatment is finished the patient may even relapse to the original state of hypersensitiveness after a few months or years. Sometimes in those who show very marked skin reactions, a prolonged course of desensitization gives the patient considerable relief from asthma or hayfever.[1]

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[1] For personal views see: Canadian Med. Assn. Jour., 1928, **19**, 171.

CHAPTER XIII.

MISCELLANEOUS EXAMINATIONS.

SALIVARY UREA INDEX (HENCH).

A Useful Method of Determining Renal Function.

Principle.—With urea retention in the body, there is an increase in the salivary urea proportionate to the increase in the blood urea. Urea has the property of forming compounds with mercury and therefore the urea retention in the body is indicated by a rise in the mercury combining power of the saliva. The mercury combining power of 100 cc. of saliva when titrated with a 5 per cent mercuric chloride solution is called the Salivary Urea Index. Normal, 30 to 50.

When mercuric chloride is added to saturated sodium carbonate a reddish-brown precipitate of mercury oxy-chloride is formed. If the mercuric chloride is first added to the saliva then a drop of the mixture added to sodium carbonate, no reddish-brown precipitate will occur until free mercury is present, that is until the mercury combining power of the saliva is entirely used up. This is a useful method of estimating function in advanced renal disease. The readings are unduly high in a few severe non-renal diseases. When patients have a dry mouth or if they are comatose, use this test adapted for the blood (page 146).

Apparatus and Reagents.

1-cc. pipet or tuberculin syringe, 5-cc. pipet or syringe.
5 test-tubes, 2 glass slides.
5 per cent mercuric chloride solution (accurate).
22 per cent sodium carbonate solution.

Collection of Saliva.—After the mouth has been thoroughly washed with one-half glass of water, a small piece of paraffin is chewed to stimulate salivary flow. A wad of paper will do but chewing gum interferes with the end-point and should not be used. Fill a test-tube to 7 cc., mark it "A," then fill a second to 7 cc. and mark it "B." Specimen "A" is used only as an approximate check as it may contain food particles, rinse water, etc., which may alter the mercury combining power. Blood sucked from the gums or muco-purulent droppings from the nasopharynx will combine with the mercury and give increased readings. Specimen "B" is used for the determination of the salivary urea index. The determination on specimen "B" should be made promptly, or at least two or three hours after its collection, to forestall bacterial action, which would cause a rise in the mercury combining power. If the specimen cannot be tested promptly, bringing it to a boil for only a few seconds will prevent bacterial decomposition.

Titration.—Two clean glass slides are placed on a sheet of paper and 12 drops of the saturated sodium carbonate are placed on the slides. Five cubic centimeters of saliva are measured into a test-tube and with a 1-cc. pipet 1.5 cc. of the 5 per cent bichloride solution. Cork the test-tube and invert several times to thoroughly mix the saliva and bichloride solution. With a clean pipet add a small drop of the mixture to a drop of the sodium carbonate solution on the glass slide. If a reddish-brown tinge promptly appears, the end-point has been reached; if no color or a canary-yellow appears the titration (3 or 4 drops at a time) should be carried further and the mixture tested. The brownish precipitate develops in about three seconds. If more than 3 or 4 drops are used, correction should be made by adding a value of 2 to the salivary urea index for each 3 or 4 test drops used. Repeat the test with specimen "B," going very slowly when approaching the end-point. The number of cubic centimeters of saliva used multiplied by 20 gives the salivary urea index. Normal 30 to 50.[1]

The probable blood urea equals 1.43 multiplied by salivary urea index minus 34. Normal blood urea is 20 to 40 mgm. per 100 cc. of blood. Normal blood urea nitrogen is 10 to 20 mgm. per 100 cc. of blood. Blood urea rises only after two-thirds of glomerular substance has been destroyed.

REACTION TO ATROPIN IN TYPHOID (MARRIS' TEST).

Principle.—The normal increase in pulse-rate following injection of atropin is absent in early typhoid fever. This is due to an antagonistic action between atropin and typhoid on the inhibitory mechanism of the heart.

The Test.—Take the pulse when the patient is at rest and digestion is completed. Then inject hypodermically $\frac{1}{33}$ grain (0.002 gm.) of atropin. After twenty minutes count the pulse every five minutes until the increase in rate begins to diminish and mark down the rates per minute as they are counted.

Interpretation.—Any increase in pulse-rate following atropin is due to that drug. In the fevers other than typhoid and in normal subjects there is an average increase of over 30 pulse beats per minute[2] and the highest rate is about thirty minutes after the injection. In typhoid or paratyphoid fever the pulse-rate increase from the fifth to the fourteenth day after the onset of the fever is less than 15. Later in the disease the reaction is not so reliable but Osler states that 3 negative reactions during the first two weeks of a fever excludes typhoid.

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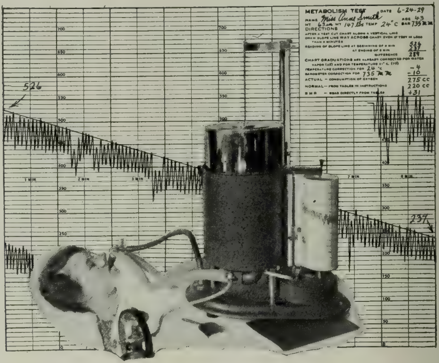
[1] With the saliva from patients with a very high urea retention, determination of the end-point is a little difficult, as a brown precipitate tends to appear slowly before the real end-point is reached. The latter may, however, be determined when a definite brown precipitate appears promptly within three seconds.

[2] Jour. Pharm. Exp. Therap., 1926, **27**, 77.

The results are not reliable in patients over fifty years of age, especially if they are arteriosclerotic. Results are vitiated by a pulse-rate over 100 or severe toxemia. The test is useful in those who have been recently inoculated with typhoid vaccine which might render the Widal test positive in the absence of typhoid.

BASAL METABOLISM.

Metabolism, popularly referred to as the fire of life, is the process by which the body turns its nutrition into energy. The metabolic rate may be determined by the rate of oxygen consumption or by the heat given off. It is greatly increased by bodily activity. A basal metabolism is secured by placing the body at complete rest. Then the thyroid gland is the main factor in varying this rate.



METABOLISM TEST DATE 6-24-29
NAME Miss Anne Smith AGE 43
HT 63 in WT 147 lbs TEMP 24° C BAR 735 MM
DIRECTIONS
AFTER A TEST CUT CHART ALONG A VERTICAL LINE
DRAW SLOPE LINE WAY ACROSS CHART EVEN IF TEST IS LESS THAN 8 MINUTES
READING OF SLOPE LINE AT BEGINNING OF 8 MIN 526
AT ENDING OF 8 MIN 237
DIFFERENCE 289
CHART GRADUATIONS ARE ALREADY CORRECTED FOR WATER VAPOR (25) AND FOR TEMPERATURE 21° C. (75)
TEMPERATURE CORRECTION FOR 24 °C -4
BAROMETER CORRECTION FOR 735 MM -10
ACTUAL — CONSUMPTION OF OXYGEN 275 cc
NORMAL — FROM TABLES IN INSTRUCTIONS 220 cc
B M R — READ DIRECTLY FROM TABLES +31

FIG. 101.—Simplified metabolism tester—"The Sanborn Grafic."

Apparatus.—Small portable machines which measure the oxygen consumption are generally used for clinical purposes. There are many satisfactory types on the market. The Benedict-Roth has many advantageous features. Models now manufactured have a recording drum for marking the depth and rate and regularity of respirations. Special directions for operation accompany each apparatus and will not be given here. They are quite easily followed. The technique is simple and anyone who uses care and manages patients kindly will obtain satisfactory results. If one can obtain readings close to zero in normal controls, the technique may be considered satisfactory.

Directions to Patient.—A preliminary test is good policy to acquaint the patient with the nature of the examination and rob the procedure of any disagreeable uncertainties. Emotional excitement increases the metabolism sometimes by 20 per cent. Make a record of the sex, age, height and weight.

The test is best performed before breakfast. The patient should eat a light supper at 6 or 7 o'clock the previous evening and eat or drink nothing further until the test is performed at 8 or 9 o'clock next morning. He may walk to the examining room but should lie on a couch perfectly still without reading or doing work of any kind for one-half hour previous to the test. Make a record of the temperature, pulse and respiration and test the apparatus for leaks.

The test is performed by having the patient breathe from an oxygen circuit attached to the lips. It is necessary to clamp the nostrils to avoid leaks. The amount of oxygen used up in six minutes of steady uninterrupted breathing is compared with the amount used by a normal person of the same age, weight, height and sex, making corrections for temperature of the spirometer and barometric pressure according to the directions accompanying the apparatus. Readings above normal are recorded as plus per cent and those below normal as minus per cent. Normal limits are from plus 10 to minus 10.

Interpretation.—The basal metabolic reading is an index of thyroid activity, a plus reading indicating over activity and a minus reading indicating subactivity. The usual factors other than thyroid which influence the metabolism are physical exertion, digestion, emotional disturbances, fever, marked heart failure or anemia and leukemia. Physical exertion is avoided by rest and digestion is brought to a standstill by the overnight fast from food. Irregularity of respiration is a guide to emotional disturbances. The thermometer indicates any fever and a blood examination will exclude leukemia which is a very rare disease except in the public wards of large hospitals.

The Apparently Normal Reading (Plus 10 to Minus 10).—A large number of readings are within 2 or 3 per cent of the absolute normal. Occasionally some individuals, however, appear to have a normal of their own which is slightly out of line with the average reading of a large number. These individuals make the interpretation of readings difficult. For instance a person who would have a normal reading of plus 10 may later on suffer from severe clinical myxedema and the metabolism rate be only minus 5. The reverse may be true when the person with a normal rate below zero develops goiter and has severe symptoms with a reading of only plus 15. Very rarely definite clinical exophthalmic goiter showing exophthalmic tremors, purposeless movements, gastrointestinal upsets, moist brownish skin, insomnia and intense muscular weakness, especially of the recti femoris, have occurred with a normal basal metabolism reading. This occurs only rarely so that if the symptoms are not typical, hyperthyroidism should not be diagnosed in the presence of a normal metabolism. Ninety-two per cent of normal individuals have a basal metabolism within plus or minus 10 per cent of normal[1] and according to DuBois 99 per cent of normals are within plus or minus 15 per cent. The basal metabolism test frequently gives valuable information in patients who are a clinical puzzle.

Low Basal Metabolic Rates.—Readings of minus 10 to 20 per cent are usually associated with milder degrees of thyroid lack and the symptoms of myxedema may not be at all typical. Such signs as pains in the muscles, fatigue and loss of energy, sensitiveness to cold and recurring bronchial infections may be present. Edema such as occurs in nephritis or heart failure will in itself give a basal metabolic reading of 20 to 30 per cent below the average normal.

Readings from minus 20 to 40 are usually accompanied by definite signs of myxedema such as slow mental processes, the patient sitting motionless for long periods of time, great desire to sleep, loss of hair on the head and the outer third of the eyebrows, a puffiness about the eyes, and thick pads of fat over the clavicles and a definite lemon tint to the skin. Occasionally patients who have marked anemia with a primary blood picture show a minus basal metabolic rate. Subthyroid patients develop disagreeable symptoms at the beginning of thyroid medication but after they have been treated until their symptoms disappear they live most comfortably when the basal metabolism is stabilized about 5 points under normal. A few, who have unaccountably low metabolism rates and feel perfectly well, experience no change in their sense of well-being when the metabolism is brought up to normal by the taking of thyroid extract. A patient with a basal metabolism rate of minus 15 per cent can usually tolerate 2 grains of thyroid extract daily. However in the matter of dosage each patient is a law unto himself and the patient's sense of well-being should determine the optimum dose rather than sufficient thyroid extract to make their metabolism conform with normal standards.

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[1] Boothby and Sandiford: Jour. Biol. Chem., 1922, **54**, 767.

Readings Above Normal.—Readings from plus 10 to plus 40 occur in adenomas of the thyroid. Colloid goiter occurs in young persons from the age of puberty to twenty-five years and is usually endemic. Most of the metabolic readings are normal while a few are elevated up to 20 per cent above normal. In adenoma there is usually a swelling of the neck for many years but the hyperthyroidism does not appear until after thirty-five years of age and the metabolism averages 30 per cent above normal. Readings above 50 usually signify exophthalmic goiter which usually occurs in patients from twenty-five to thirty-five years of age. When the metabolism is less than plus 30 per cent, symptoms are usually absent in exophthalmic goiter. Surgical operations on patients with basal metabolism rates of 40 to 50 per cent above normal are attended by considerable risk especially if the myocardium is damaged. Iodine medication in the form of Lugol's solution causes a rapid decrease in the metabolic rate so that in a week it is only one-quarter of what it was formerly. After about a month even when medication is continued the rate mounts and the symptoms return if the goiter has not been removed surgically. Fever, pernicious anemia and leukemia give an increased resting metabolism and should be ruled out before making a diagnosis of exophthalmic goiter. Increased perspiration, especially of the hand, and an increase in the basal pulse-rate almost always accompany an increased metabolic rate. They may also occur in other conditions however.

Relation of the Pulse to the Basal Metabolism.—In subthyroid patients the pulse is usually between 60 and 70. The basal metabolism is seldom abnormally high when a pulse-rate taken with the patient at complete rest, is below 85 per minute. The basal pulse-rate usually increases as the basal metabolic rate increases so that a basal pulse of 100 or more usually accompanies a basal metabolism of plus 40 per cent and a basal pulse of 140 goes with a basal metabolic rate around plus 75 per cent.

Because the basal pulse-rate in individuals is frequently different changes from normal in a patient's pulse-rate taken at complete rest is a better indicator of his metabolism than deductions made from a given pulse-rate. When a satisfactory metabolic reading is taken the progress of the patient may be adequately followed for a period of time by noting the basal pulse-rate instead of doing frequent metabolic estimations.

There are several important causes of tachycardia other than an increase in metabolism, however. They are sinus tachycardia occurring in psychoneurotic individuals or during nicotine poisoning, and physical or nervous exhaustion as well as paroxysmal tachycaria and organic diseases of the cardiovascular system.

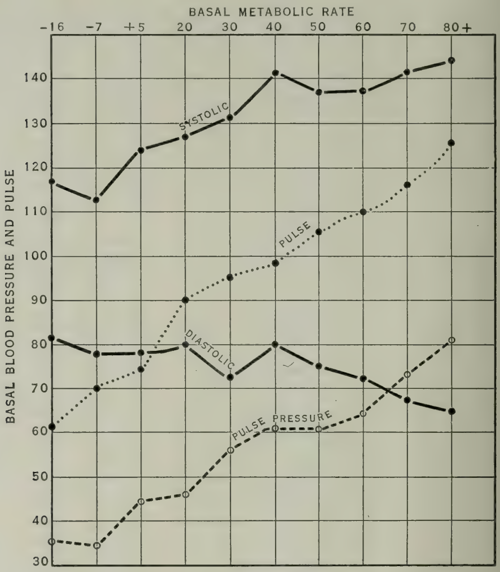

FIG. 102.—Relation of the average basal metabolism to the average basal pulse rate and blood-pressure in a series of 252 patients suspected of thyroid disturbances. 110 were normal, 121 increased and 21 subnormal metabolism readings. With any given pulse-rate, a few metabolic readings varied 20 points from the average. (Investigation by Dr. J. D. Adamson.)

RAPID MICROSCOPIC TISSUE EXAMINATION (TERRY).

This method by its simplicity and rapidity opens up the wealth of information to be obtained by cellular study of diseased tissue and enables the surgeon to make a microscopic diagnosis during or immediately after the removal of a mass of tissue. Study of illustrations in the best texts in pathology and surgery and review of permanent sections will qualify the surgeon to interpret microscopic pictures.

PRINCIPLE.—If a thin slice of tissue which is stained on one side only, be examined microscopically in the wet state with the stained side uppermost, it shows definite cellular detail.

Apparatus and Reagents.—A microscope and lamp (60 Watt) with frosted white bulb is best, a sharp razor, a slab of cork or thick cardboard about 6 inches by 6 inches, common pins, pointed forceps, 2 medicine droppers, 1 small camel's hair brush, clean cover-glasses and microscopic slides, 2 tumblers of water, neutral polychrome methylene blue (prepared by the National Aniline and Chemical Company, 40 Rector Street, New York City). As stains ordinary methylene blue or hematoxylin are good substitutes.

Choice of Material.—Either the fresh unfixed tissue or tissues fully fixed for twenty-four hours or more in 10 per cent formalin may be examined. The fresh tissues are softer and the razor must be very sharp or it will displace the cells. Handle the sections carefully in staining, washing and mounting to avoid loss of cells. Fixed tissues give excellent results but must be cut quite thin because the sections are not so translucent as in the fresh state.

Any tissues containing calcium deposits cannot be properly cut. When in the fresh state tissues that are very soft, friable, necrotic or hemorrhagic are unsatisfactory. It is difficult to section and stain uterine curettings by this method. Tissues of smooth muscle may by its contractions throw the section into folds. Soft tissue may be rapidly fixed by immersing for a minute a small block in formalin that is just below boiling-point. Very satisfactory sections may be made from fresh breast tumors, lesions of the cervix uteri and intestine or in fact any tissue of firm or fibrous consistency.

Preparation of the Tissue.—*Selection.*—Make a section directly through the tumor mass and after careful inspection and palpation excise a block to be examined, preferably including the margin of the tumor. Use a very sharp knife for this and handle the tissues lightly to avoid displacing cells. A convenient size is $\frac{1}{2}$ by $\frac{1}{2}$ by $\frac{1}{4}$ inch. Pin this block to the cork slab or cardboard plate, using two or more pins. The tissue should not move while it is being cut so it is necessary to pin it down near the side from which the slice is taken. If the block consists mainly of soft tissue with one side of tough material place the softer portion upward so that it will not be crushed in cutting through the denser tissue.

Making the Section.—By placing a drop of water on the section and immersing the razor in the tumbler the friction in cutting is reduced and it is possible to obtain thinner slices without disturbing the tissue cells. To cut the slice rest the point of the razor on the cork plate about 2 inches to the left of the immobilized wet tissue and the heel of the blade at the top of the tissue block. Without raising or lowering the blade draw it to the right so that one complete stroke severs the tissue. Do not press the razor into the tissue which would squeeze out cells. One stroke cuts the first piece off about $\frac{1}{8}$ inch in front of the pins. This section is usually discarded and with a similar stroke a second very thin slice is cut and placed in water. The section should be about $\frac{1}{32}$ of an inch thick. The fresh translucent tissue may be cut somewhat thicker.

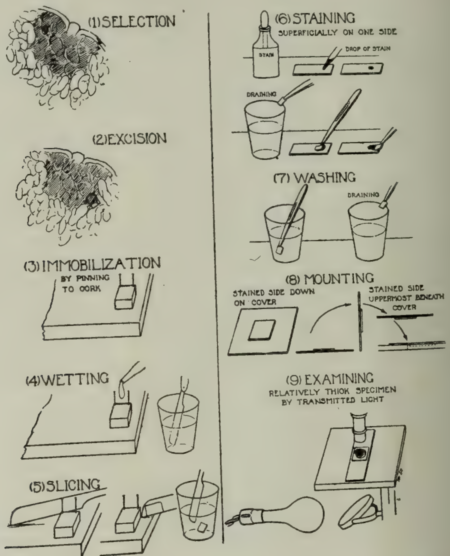
(1) SELECTION
(2) EXCISION
(3) IMMOBILIZATION
BY PINNING TO CORK
(4) WETTING
(5) SLICING
(6) STAINING
SUPERFICIALLY ON ONE SIDE
STAIN
DROP OF STAIN
DRAINING
(7) WASHING
DRAINING
(8) MOUNTING
STAINED SIDE DOWN ON COVER
STAINED SIDE UPPERMOST BENEATH COVER
(9) EXAMINING
RELATIVELY THICK SPECIMEN BY TRANSMITTED LIGHT

FIG. 103.—Nine steps in preparing the tissue for microscopic examination. (Terry: Jour. Lab. and Clin. Med., 1929, **14**, 510.)

Recently Terry has advised the following technique:[1]

Place the wet section on a glass slide, blot up any excess of water from the surface of the section and apply a film of polychrome stain with a camel's hair brush or rod of any kind for two or three seconds.

Ordinary Loeffler's methylene blue gives a clear, cut microscopic picture in ten to fifteen seconds but it stains connective tissue a pale blue whereas the polychrome stains it pink. Hematoxylin requires thirty to sixty seconds to stain and should be applied on a filter paper. After staining wash the section thoroughly by spurting water from a medicine dropper over it several times. Leave a drop of clean water around the section and apply a cover-glass over the stained surface. Using artificial light examine under the low-power lens of the microscope.

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[1] Jour. Lab. and Clin. Med., 1929, **14**, 519.

If the light is strong the polychrome fades out rapidly and if the section is restained several times the deeper cells absorb too much stain and it may be necessary to stain another section to complete the examination. To keep the section for future observation wash off the stain with alcohol and preserve in 10 per cent formalin. It can be restained when necessary or submitted for permanent paraffin section.

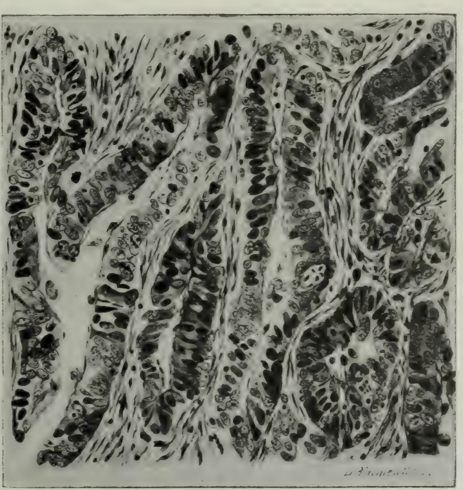

FIG. 104.—Razor section of an adenocarcinoma of the stomach. Grade 3 Broders. (Terry's stain, Jour. Lab. and Clin. Med., 1929, **14**, 510.)

Examination.—Artificial light is better than daylight. A 60 Watt frosted bulb is best. The mirror should give a maximum illumination and the diaphragm be adjusted to give a moderate lighting. Moving the condenser sometimes improves the picture. The microscopic appearance resemble very much a good frozen section. The low-power lens will give most information by showing the general relation of cells. Occasionally the high-power lens is of value to confirm various cellular outlines. Epithelium and nuclear material of all kinds stain a dense blue, connective tissue is a reddish-purple and red blood cells a yellowish-green.

BIOPSY—THE REMOVAL AND TRANSPORTATION OF TISSUES FOR MICROSCOPIC DIAGNOSIS.

Small superficial particles of tissue are worthless. If there is an ulcer the most information regarding malignancy is derived from a vertical section into the base. The size of the section will vary with the lesion. It should be at least $\frac{1}{4}$ inch deep, $\frac{1}{4}$ inch long and $\frac{1}{16}$ to $\frac{1}{8}$ inch thick.

Formalin, 5 per cent is an ideal preservative and fixative. The tissue should be immersed in four to five times its volume of the solution. When large surgical specimens are sent they should be incised to aid the penetration of formalin and the volume of solution should be several times that of the specimen. A short clinical history should accompany each specimen and an exact description or sketch of the location from which the specimen was taken. This will enable the pathologist to render a more intelligent and helpful opinion.

Methods for the collection and transportation of specimens of blood are to be found prior to the description of technique for the various tests.

TESTS FOR POISONS IN URINE, GASTRIC AND COLONIC CONTENTS.

If the result of these examinations is to be used in a court of law, the examination must be done by an expert chemist or it will be of no value.

When a practitioner suspects that a death has resulted from poisoning he should consult the coroner and if permission for a post-mortem is obtained, the stomach, tied at both ends, should be placed in a *clean* fruit sealer. If the death has not occurred, it is best to collect urine and colonic contents in similar containers. It is very important that the containers should be handed to an officer of the law who will convey them in person to the chemist. Mailing or expressing the material may raise the question of substitution.

If the practitioner wishes to do preliminary tests the reactions for alcohol in toxic quantity, atropine and strychnine are easily carried out as follows:

Alcohol in Toxic Quantity.—The odor is characteristic.

Atropine.—Evaporate the solution to a few drops. Filter and place in any animal's eye. Dilatation of the pupil is positive for atropine.

Strychnine.—To the partially evaporated solution add 1 or 2 drops of concentrated sulphuric acid on a white plate. Then add a small crystal of potassium bichromate. A deep blue color changing to violet, purple, cherry-red, and finally to orange is positive for strychnine.

Tests for arsenic, morphine, lead and mercury involve special equipment and reagents so it is best to have an analytical chemist perform these examinations.

CHAPTER XIV.

THE LABORATORY EQUIPMENT.

The laboratory equipment is a most important detail. It is a necessary outlay in order to obtain the quick information on the analysis of urine or blood which we so much desire during the course of the examination of a patient.

Much time and though has been spent on the selection of the equipment recommended. The procedures chosen are those which, in the writer's opinion, combine the greatest degree of accuracy and simplicity. The materials utilized are low in cost and the reagents will not deteriorate.

The microscope is the greatest outlay. The number of reagents has been cut down to a minimum. Where possible a common reagent or apparatus is used for as many procedures as possible. A 1 pound bottle of glacial acetic acid can be diluted as a fluid for leucocyte counting, as a reagent in testing for albumin and in the chemical test for occult blood.

Except for the preparation of the simpler reagents such as acid alcohol and dilutions of concentrated reagents, it is best for the practitioner to buy them all ready prepared. The local druggist can supply most of them. The stains and indicators, decinormal sodium hydroxide and Benedict's quantitative solution for sugar testing had better be purchased at a large laboratory supply house unless the volume of work done is sufficiently large to justify making up the reagents in the laboratory.

Apparatus may be obtained through local medical companies and from such firms as the Fisher Scientific Company, Pittsburgh, Pa., or the A. H. Thomas Company, West Washington Square, Philadelphia, Pa.

A flat-topped desk makes a very compact laboratory, each drawer containing all the reagents required for one or more tests. The best surface is the black acid-proof stain.

Solution No. 1.

Copper sulphate 25 gm.
Potassium chlorate (or permanganate) 25 gm.
Water 200 cc.

Solution No. 2.

Anilin oil 24 cc.
Hydrochloric acid concentrated 36 cc.
Water 200 cc.

Sandpaper and plane any varnish off the surface. Apply two coats of Solution No. 1 hot, then two coats of Solution No. 2, without heating allowing each coat to dry before the next is applied. After the last is dry, rub with a clean cloth to remove excess of the stain and then rub in thoroughly a mixture of equal parts of turpentine and linseed oil. To keep the surface in good condition rub it weakly, using a cloth saturated in the turpentine-linseed mixture.

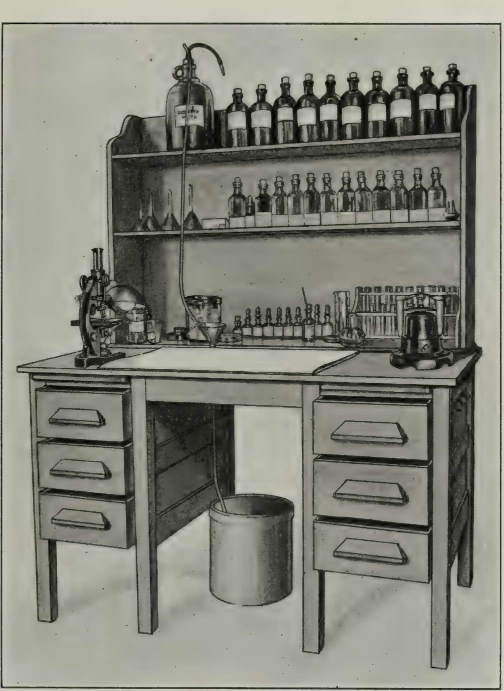

Fig. 105.—A flat top desk with shelves at back, equipped as a minimum laboratory. A bench at the side containing a sink should be the next extension.

Two shelves at the back will hold all the necessary reagents. Place summaries of the various tests on a near-by wall in ready view.

It is of the utmost importance to have a liberal supply of all reagents on hand and to see that the equipment is in good condition. The results of a clinical test are required quickly and if the reagents are not readily available much valuable time is lost or the test is not done. A quantitative estimation of the amount of sugar in a diabetic urine can be done in five minutes but if supply of one of the reagents is used up, a week may pass before the estimation can be made.

The potency of reagents should be tested occasionally by control tests, although the tests outlined here use, as far as possible, reagents that do not deteriorate. What a shock it is to find after doing occult blood tests for months with negative results that a drop of blood from your own finger diluted in water gives a negative result.

In doing leucocyte counts, if the blood pipet is not wiped off before immersion into the diluting fluid, the cells fall to the bottom of the bottle and after many counts a sediment may accumulate. If the pipet is then dipped to the bottom, the cells will be drawn in with the diluting fluid and the count very much higher than it should be. Absolute cleanliness is very essential to accurate results.

In the heat test for albumin in urine, a not too clean test-tube may give the appearance of a faint trace when none is really present. The bottom of the centrifuge tube should be carefully examined to see that no sediment remains after the tube is cleaned. After an ordinary washing the pus from a urine may remain adhered to the bottom of the tube and if a normal urine is next centrifuged it will appear to contain pus.

By keeping an order sheet posted nearby it is easy to make a note of any reagents nearly used up; then by spending half an hour every week or two the reagents can be rechecked and a new supply ordered before the old one is entirely exhausted. Only by such a method can the laboratory be kept to a point of high efficiency.

The following may be used as a list to check over the supply of apparatus and reagents:

Apparatus.—The list of apparatus, reagents and stains for routine use includes many articles such as glass syringes, needles, trocars, rubber gloves, etc., which a practitioner would have for his other medical work. The list of special equipment is very full. Because there are many different fields of medicine even a group of practitioners would rarely use every item. By purchasing powdered chemicals and making up the reagents the cost could be much reduced. The cost would also vary according to the grade of equipment desired.

Routine.—Microscope with (1) oculars × 5 and × 10 (2) objectives 16 mm. and 4 mm. dry and 1.9 oil-immersion lens and simplified mechanical stage.

Electric lamp with frosted bulb or coal-oil lamp.

Sahli hemoglobinometer with tinted solid glass standard.

Hemocytometer. Neubauer ruling, preferably with Bureau of Standards stamp.

Spring blood lancet. A pen nib with one point broken and the other sharpened chisel-like or a hypodermic needle, 22-gauge, makes a cheaper but much less satisfactory substitute.

1 gross glass slides 1 by 3 inch, best quality.

1 slide box for 100 slides.

1-ounce No. 2 cover-glasses 22 mm. square.

200 labels.

2 dozen test-tubes $\frac{3}{4}$ by 6 inches.

2 dozen test-tubes $\frac{5}{8}$ by 5 inches, thin glass for heating.

2 test-tube brushes.

1 double rack to hold 12 test-tubes.

6 3-inch funnels.

1 packet medium filter paper.

1 urinometer with long stem. (See page 287.)

3 conical urine glasses.

Centrifuge, preferably an electric one capable of 2000 or more revolutions per minute.

12 centrifuge tubes, 15 cc.

1 glass marking pencil.

Esbach's albumin percentage tubes.

2 quart-sized fruit sealers.

1 Ewald bulb.

1 Rehfuss gastric or duodenal tube.

1 gastric tube $\frac{3}{8}$ inch diameter.

1 LaMotte outfit for estimation of the hydrogen-ion concentration in gastric contents.

2 16-ounce measuring glasses.

2 lumbar puncture needles 22-gauge and 3 to $3\frac{1}{2}$ inches long, preferably gold but nickel will do.

Manometer. Stopcock attached to 2-mm. glass rubber tubing (see page 237).

Millimeter tape or rule.

1 quart-sized thermos bottle.

1 bath thermometer.

12 tubes of Loeffler's blood serum in sealed tubes.

1 microme loop and handle for inoculating needles.

12 blood vacuum tubes (Keidal).

12 blood vacuum tubes (Keidal) containing 100 mgm. of sodium fluoride as anticoagulant and preservative.

Glass syringe, 10 cc.

22-gauge needle 1¼ inches long.

Water-bath heated by electric plate or spirit lamp with tripod, if gas is not available.

1 trocar gauge, 12 to 15.

2 1-cc. tuberculin syringe.

Absorbent or gauze sponges.

Tourniquet, 18 inch of ¼-inch rubber tubing.

5 feet glass tubing, 2 mm. internal bore, glass file.

6 heavy rubber bands.

3 pipets, 5 cc.

5 pipets, 1 cc.

1 pipet, 10 cc.

2 25-cc. all glass syringes.

2 pairs rubber gloves.

6 Petri dishes.

1 box toothpicks.

6 sterile swabs.

2 100-cc. graduated cylinders.

1 paper of pins.

6 medicine droppers.

Reagents.—*Routine.*—Acid acetic, glacial, 1 pound.

Acid acetic, 2 per cent for leucocyte count (see page 21), 8 ounces.

Acid acetic trichlor, chemically pure, 1 ounce.

Acid hydrochloric tenth-normal (accurate), 8 ounces.

Acid hydrochloric concentrated, 8 ounces.

Acid nitric concentrated, 1 pound.

Acid sulphosalicylic 3 per cent (see page 275), 2 pounds.

Anesthetic, local, in tablets or ampules.

Ammonium hydroxide concentrated, 8 ounces.

Ammonium sulphate saturated solution (see page 247), 4 ounces.

Blood grouping serum 1 cc. or 12 capillary tubes each of Group A (2) and B (3.)

Benedict's copper sulphate solution for qualitative sugar test (see page 275), 2 pounds.

Benedict's copper sulphate solution for quantitative sugar test (accurate) (see page 310), 8 ounces.

Benzidine powders (accurate) for occult blood (see page 323), 12.

Canada balsam, 1 ounce.

Oil of cedar, 1 ounce.

Chloroform, 4 ounces.

Esbach's picric citric acid reagent for quantitative albumin test (see page 310), 4 ounces.

Ferric chloride aqueous 5 per cent for lactic test, 1 ounce.

Ferric chloride aqueous 10 per cent for diacetic test, 8 ounces.

Hayem's solution for red cell counts (page 29), 8 ounces.

Hydrogen peroxide, 8 ounces.

Iodine, tincture of, 4 ounces.

Litmus blue, 1 vial.

Mercuric chloride 5 per cent (accurate), 4 ounces.

Phenolphthalein indicator 1 per cent in 90 per cent alcohol, 1 ounce.

Potassium oxalate 20 per cent solution, 1 ounce.

Sodium carbonate anhydrous, chemically pure, 4 ounces.

Sodium carbonate 22 per cent solution, 1 ounce.

Sodium citrate, chemically pure, 20 papers, each containing 3 gm. (see page 73).

Sodium chloride, chemically pure, 4 ounces.

Sodium fluoride, 12 papers, each containing 100 mgm. for preserving 5 cc. of blood (see page 136).

Sodium hydroxide tenth-normal (accurate), 1 pound.

Sodium nitroprusside crystals, 1 ounce.

Sodium oxalate, chemically pure, 1.4 gm. in papers (see page 69), 1 ounce.

Silver nitrate 10 per cent, 2 ounces.

Talcum powder, 1 ounce.

Toluol, 1 ounce.

Vaseline, 1 ounce.

Water, distilled, 1 gallon.

Xylol, 8 ounces.

Stains (prepared solutions preferably in dropper bottles).—*Routine.*—Methylene blue, two 1-ounce containers. (See page 203.)

Carbol fuchsin (Ziehl-Neelson), two 1-ounce containers. (Page 224.)

4 ounces acid alcohol (3 per cent HCl. in 95 per cent alcohol).

Wright's stain, two 1-ounce containers (see page 40).

Delafield's hematoxylin (see page 39).

0.5 per cent eosin in absolute methyl alcohol (see page 39).

1 ounce crystal violet (see page 203).

1 ounce Gram's iodine.

1 ounce safranin 1 per cent aqueous.

Special Apparatus.

- Albumin standards for rapid quantitative test. Kinsbury Clark. (See page 280.)
- Phenolsulphonephthalein renal function test standards. (See page 306.)
- Hunt micro blood sugar outfit.
- Marriott apparatus for estimating the alveolar air carbon dioxide tension.
- Bromsulphthalein standards. (Hynson, Westcott & Dunning.)
- Simplified Van Slyke for estimation of CO_2 in blood. (Emil Greiner Company.)
- Van Slyke and Cullen standard blood urea apparatus.
- Suction pump connected to a water-tap.
- Four Folin-Wu blood sugar tubes.
- Two 2-cc. Ostwald pipets.
- Two 50-cc. Erlenmeyer flasks.
- Micrometer eyepiece (in which 1 division equals a micron under the oil-immersion lens).
- Hematocrit (Van Allen) with cap.
- 2 15-cc. graduated centrifuge tube.
- Klett colorimeter with Newcomer hemoglobin standard and Killian icterus index standard, or
- 2 macro and 2 micro Cutler pipets.
- Camera, preferably postcard-size kodak.
- 1 sheet artist's copying paper.
- 1 volumetric flask, 100 cc.
- 1 volumetric flask, 500 cc.
- Two 30-cc. Luer syringes with 2 needles 1½ inches long, gauge 18 and 20.
- Doremus ureometer.
- 1 sharp razor.
- Hair tweezers.

Special Reagents.

- Adrenalin chloride, 1 ounce.
- Aniline oil, 1 ounce.
- Bromsulphthalein, 6 ampules.
- Calcium chloride, chemically pure, 4 ounces.
- Diazo reagents 1 ounce each. (See page 315.)
- Obermeyer's reagent (see page 315), 4 ounces.
- Petrolatum liquid, 4 ounces.
- Paraffin in alcohol (see page 248), 4 ounces.
- Phenolsulphonephthalein ampules for test of renal function, 12 ampules.
- For Quantitative chloride test in urine,
  - Standard (accurate) silver nitrate and ammonium sulphocyanate solutions (see page 314).
- For Creatinine in blood,
  - Creatinine standard solution (see page 150), 4 ounces.
  - Picric acid certified chemically pure, saturated solution (2 gm. per liter).
  - Sodium hydroxide 10 per cent, 4 ounces.
- For Standard blood sugar, Folin-Wu,
  - 10 per cent sodium tungstate, 1 pound.
  - Two-thirds normal sulphuric acid, 1 pound.

  - 1 per cent accurate dextrose in 0.25 per cent benzoic acid for standard, 4 ounces.
  - Alkaline copper sulphate solution (see page 137), 1 pound.
  - Molybdate phosphate solution (see page 137), 1 pound.
  - Glucose 50 per cent for tolerance test, 1 pound.
- For Standard blood urea test, Van Slyke and Cullen,
  - 100 urease tablets.
  - Antifoam solution (see page 145), 4 ounces.
  - Potassium carbonate, chemically pure, 2 ounces.
  - One-fiftieth-normal hydrochloric acid, 1 pound.
  - One-fiftieth-normal sodium hydroxide, 1 pound.
  - 1 per cent alizarin indicator, 1 ounce.
- For Urea Concentration Test,
  - Urea, chemically pure, 4 ounces.
  - Hypobromite reagents (see page 308), 1 pound each.
- For Urobilin Test,
  - Zinc acetate, saturated solution in alcohol, 1 pound.
  - Calcium chloride 10 per cent aqueous solution, 2 ounces.
  - Sodium hydroxide 25 per cent (see page 336), 1 ounce.

Biologicals.

- Antityphoid vaccine, 10 cc.
- Typhoid culture formalinized for Widal, 1 ounce.
- Dick test outfit (see page 343).
- Schick test outfit (see page 338).
- Scarlet fever antitoxin for Schultz Charlton reaction (see page 344).
- Smallpox vaccine (see page 345).
- Serums for typing pneumococcus Types 1, 2 and 3, 5 cc. each (see page 229).
- Hemostatic of brain emulsion, often marketed thromboplastin, 10 cc.
- Tuberculin (Koch's old), 1 cc.
- Proteins and pollens to test cutaneous reaction (see page 352).

Special Stains.

- 5 gm. brilliant cresyl blue.
- Rees and Ecker's fluid (see page 73).
- Peroxidase reagents (see page 68).
- 1 ounce Cerquair's modification of Fontana stain for spirochetes mordant (see page 233).
- 1 ounce Terry's neutral polychrome methylene blue stain.
- 1 ounce Sato and Sekiya peroxidase reagents (see page 67).

CLEANING GLASSWARE.

The method employed to clean a few glass slides, coverslips or test-tubes for ordinary use is soap and water followed by rinsing and wiping. For glass slides or coverslips a grit soap like Bon Ami works well. After rubbing on the soap paste allow it to dry and polish the surface with a dry cloth. The slides should afterward be rinsed in alcohol to remove any alkali that remains.

To clean a large quantity of glassware it should be dealt with as follows:

1. *New Glassware.*—Most new glassware is covered with a whitish deposit. A solution of 1 per cent hydrochloric, nitric or sulphuric acid in a 75 to 90 per cent alcohol solution or the sulphuric acid bichromate cleaning solution is best to remove this coating of whitish material.

After five minutes of this, rinse well in running water and finally in distilled water if absolute transparency is desired.

Test-tubes and other containers should be placed mouth down on racks to dry.

Glass slides should be immersed in clean concentrated alcohol and quickly dried with a clean cloth. Coverslips should be treated in the same manner. When drying the coverslip rub both sides simultaneously to avoid breakage. Clean dry slides and coverslips should be kept in a Petri dish or other covered receptacle ready for use. Just before use they may be passed through a flame to make the surface perfectly free from grease. If coverslips are exposed to the flame for more than a moment they will warp.

2. *Slightly Stained Glassware.*—Wash in soda or soap solution using a brush to remove particles and then rinse thoroughly in tap-water and drain dry. If cleaning is not easily accomplished do not spend time scrubbing but put the glassware in the cleaning solution.

3. *Glassware Containing Infectious Material*, should be boiled in a 2 per cent solution of sodium carbonate or washing soda for fifteen to thirty minutes or longer, then rinsed well in running water and finally in distilled water before drying. If they are still dirty they should be immersed overnight in cleaning solution.

Never boil glass in a soap solution. It leaves a very adherent whitish deposit on the glass.

4. *Glassware Smeared with Stains, Oil, Balsam or Other Substances Not Easily Removed.*—When coverslips are mounted on slides with balsam, heat over a flame to soften the balsam and slide the coverslip off. Then treat slides with an acid alcohol solution containing 1 per cent acid in 90 per cent alcohol for several hours. After this the slides should be wiped once or twice with a cloth to remove the balsam and most of the stained material and then they should be put into cleaning solution overnight.

Other glassware such as test-tubes should be washed with tap-water to remove any free material then left five to ten minutes or longer if necessary in the following sulphuric acid bichromate mixture.

Sulphuric acid, concentrated	500 cc.	or	16 oz.
Potassium bichromate	100 gm.	or	3 oz.
Water to	1 liter	or	1 qt.

This solution is very caustic to the skin. It should be always kept in a glass container or a crock and may be used many times. After glassware has stood in this solution overnight, the solution should be poured off into another container for future use and the glassware undergoing the cleaning process may be rinsed with running water in the original container and then finally with distilled water. Afterward place test-tubes bottom up on a rack to dry and immerse the slides in clean concentrated alcohol and wipe them dry with a clean cloth. It is questionable whether the time and breakage involved to clean coverslips that have been covered with balsam and oil, makes it worth while or whether it is better to discard them.

Freshly Distilled Water.—When a small amount of freshly distilled water is required it can be readily prepared without a special distilling apparatus.

Heat a piece of glass tubing and bend it into a U-shape with one long and one short arm. Insert the short arm into a cork which is placed in a flask or large test-tube containing water to be boiled for the distillation. Place a clean test-tube over the other end of glass tube. Immerse it in a vessel of cold water. This will cause the steam which passes through the glass tube to condense on the cool walls of the test-tube. Empty out the tube after the first dram has condensed. This gets rid of any foreign substance that may be in the glass tube or test-tube. Ten to 20 cc. may be distilled in this way in about twenty minutes.

STERILIZATION.

General Remarks.—Heat is the best means of sterilization. Moist heat is more efficient than dry heat. Moist heat under pressure as in a pressure boiler or autoclave is our most efficient sterilization.

It is frequently necessary to employ dry heat. Steam would liquefy a dry crystalline chemical. Test-tubes, Petri dishes and lumbar puncture needles are best sterilized in a hot-air oven. It must be kept at 170° C. for one hour to obtain absolute sterilization. If the door be opened to hasten cooling the thick glass may crack. Chemical germicides may be used where heat is not convenient or desirable. Heat may not be at hand for sterilizing syringes or pipets. It injures the temper and edge of knives. Contaminated table tops, apparatus, or hands have to be treated with germicides and for this reason it is well to know the germicidal power and characteristics of the solution used.

HEAT.—1. Steam at 126° C. in a pressure boiler kills all microbes and spores in fifteen minutes.

2. Boiling water for one minute kills all pathogenic germs but not spores.

3. Dry oven heat is best for laboratory glassware, 170° C. for one hour will kill all germs and spores.

CHEMICAL.—1. Carbolic acid, 5 per cent or cresol compound, 2 per cent, will kill microbes but not spores in one minute. Suitable for skin or instruments.

2. Biniodide of mercury 1 in 1000 is an excellent germicide but albumins greatly inhibit its action. It is more efficient for skin in a 25 per cent alcoholic solution.

3. Alcohol, 70 per cent, has merit as a good grease solvent and cleaner and is a fair germicide. Solutions stronger or weaker than the above are more inefficient as germicides.

Cleanliness of instruments and of hands makes chemical germicides more efficient.

Autoclave or Steam Pressure Sterilization.—A single exposure for fifteen minutes in an autoclave or pressure boiler is sufficient to destroy all microbes and their spores provided the temperature is 126° C. It requires at least ten minutes longer to sterilize a liter than it does to sterilize 10 cc. Where possible the material to be sterilized should be put in small containers. All autoclaves should be equipped with reliable thermometers and the temperature rather than the pressure should be the guide. A temperature of 126° C. is obtained at a pressure 20 pounds per square inch. If the autoclave be full of air the sterilization will be imperfect even if the temperature is at 126° C. A small open jet is placed in the bottom of the newer types which evacuates the residual air and allows the quickest and most efficient sterilization by the action of saturated streaming steam.[1]

In a double-walled autoclave the contents may be dried by producing a vacuum for two or three minutes, then leaving the steam in the surrounding chamber for several more minutes. If the vacuum be not used, steam in the outer chamber but cut off from the inner chamber will dry the articles in a slightly longer time. This type costs $200 to $500 but will sterilize and dry out dressings and bandages. A small single-walled pressure cooker may be obtained for about $25 that will sterilize equally as well as the expensive autoclave but it will not dry out the material sterilized. Drying is not necessary for glass utensils, culture media and laboratory apparatus. Bottles containing material to be sterilized should have their mouths covered by several layers of gauze held in place by an elastic band. The cork should be placed in the gauze and not inserted in the bottle until after sterilization. If the gauze is secured by a rubber band as a covering over the cork and mouth of the bottle it will prevent contaminating organisms gaining entrance between the bottle mouth and the cork when part of the contents have to be frequently removed. Pipets, test-tubes and Petri dishes should be wrapped in paper.

The Sterilization of Gauze and Absorbent Cotton.—Exhaust the air and then force steam through the dressings and maintain it for twenty minutes at 126° C. Exhaust by vacuum for twenty minutes. This dries the dressings after the steam pressure has sterilized them.

Low Temperature Sterilization.—Protein solutions such as vaccines cannot be boiled but may be sterilized by heating to 65° C. for one hour. If the hay bacillus (Bacillus subtilis) be present as it may be in cultures taken from the nose, the vaccine will have to be discarded because heating to 65° C. for one hour daily on ten successive days will not prevent its growth.

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[1] Benton and Leighton: Actual Temperatures Attained by Mediums in Autoclave Sterilization, Jour. Inf. Dis., 1925, p. 353.

Separating Bacteria from a Solution by Filtration.—Protein solutions for inducing tolerance in hypersensitive asthmatic patients may be sterilized by passing them through a Pasteur, Chamberlain or Berkefeld filter, which is a porous stone cylinder. This allows solutions but not ordinary bacteria to pass through.

The stone filter is put into a rubber cork which is placed in an Erlenmeyer flask with a side neck to which a suction pump may be attached. Sterilize the filter and flask under steam pressure. Pour the solution which is to be sterilized, over the stone cylinder and make a partial vacuum in the flask with the suction pump. Remove the filtrate with a sterile pipet and culture a drop to see that no bacteria are present. Occasionally the stone has a flaw and may let through pyogenic bacteria. The new Seitz filters which contain porous, renewable disks are more satisfactory than the porous stone candles.

CHEMICAL GERMICIDES.

The following chemicals will kill Bacillus coli in a WATERY solution in two and one-half minutes:

Mercury bichloride 1 in 100,000.
Mercuric potassium iodide in 1 in 100,000.
Alcohol 70 per cent.
Carbolic acid 1 in 80.
Lysol 1 in 150.
Liquor cresol saponatus 1 in 200.
Mercurochrome 1 in 2,000.
Acriflavine 1 in 20 did not kill Bacillus coli.
(Serum greatly enhances its action).
Gentian violet 1 in 500.
Malachite green 1 in 1000.
Iodine 1 in 50,000.
Silver nitrate 1 in 2,000.
Dakin's hypochlorite solution 1 in 20,000.
Chlorine 1 in 75,000.
Formaldehyde 2 per cent (equals 5 per cent formalin).
Thymol 1 in 1,500.
Boric acid, saturated solution which is 1 in 25 did not kill Bacillus coli.

Each of these substances has its disadvantages. The soluble mercury solutions, although they are very powerful, are irritating to tissues and do not penetrate albumin substances. The phenols, although they penetrate skin and albumins well, have an unpleasant odor. Mercurochrome, acriflavin and iodine, although they are non-irritating and penetrate the skin well, produce an unsightly stain. The hypochlorite solutions are efficient in open wounds on account of their antiferment rather than their germicidal properties.

Special Properties of Some Common Germicides.—*Mercuric Potassium Iodide* (*Biniodide of Mercury*) 1 *in* 1000.—This substance possesses all the advantages of bichloride of mercury and it is not so readily precipitated by pus or albuminous substances. It is less irritating to the skin than mercuric chloride and it will not corrode steel or nickel instruments over a period of two or three hours. While it has a high germicidal power this is lessened by 90 per cent on contact with albuminous fluids such as muco-purulent discharges and excreta in contrast to the phenol group of germicides which are inhibited little if any by albumins. For hands it is best to make up 1 in 1000 biniodide in a 25 per cent alcoholic solution to aid penetration into the oily skin.

Tablets are marketed containing mercury biniodide and a small amount of pink or green dye. One tablet to a pint of water makes a 1 in 1000 solution (7 grains in a U. S. pint of 16 ounces or 8.75 grains in an Imperial pint of 20 ounces make approximately 1 in 1000).

As the amount of dye is constant in the tablet any gross mistake in the preparation can be noticed by a change in the depth of color of the solution.

Carbolic Acid 5 *Per Cent.*—This is a good antiseptic for general use. It penetrates albuminous substances moderately well and its odor is not strong. Most hands can stand 5 per cent for a short period if not used too frequently and very few have any trouble with 2.5 per cent when used continually. The chemically pure carbolic is in solid form. Warm it and add 1 part of water to 9 parts of crystals to keep it liquid which is the same strength as the commercial "pure carbolic." Make up the 1 in 40 or 1 in 20 with warm water, Lord Lister, pioneer in modern surgery used carbolic in 5 and 2.5 per cent strengths to prevent infection of clean wounds and with great success.

Cresol.—Cresol is not very soluble but when mixed with equal parts of soft soap to make the *Compound Liquor of Cresol* it is about three times as efficient as carbolic acid and has agreeable soapy properties. Its odor is unpleasant. A 1 per cent solution in soft or distilled water makes an ideal and economical solution for hands or apparatus. It penetrates albuminous and fatty material well and kills all pyogenic bacteria in one minute. *Three drams to* 1 *quart makes a* 1 *per cent solution.*

There are many proprietary preparations of liquor cresol composites. One is lysol, a refined liquor cresol compound which is widely advertised. Some proprietary preparations contain alcohol which makes them less bactericidal than compound cresol itself but usually they are two to three times as efficient as carbolic.

Hexylresorcinol 1 *in* 1000.—This is one of the newer germicides which is relatively non-irritating and non-toxic and stated to be forty-five times as efficient as phenol. When taken internally it renders the urine bactericidal. It has no odor and leaves no stain. Its germicidal efficiency is partly due to its low surface tension. It destroys bacteria in less than fifteen seconds but up to the present it is quite expensive.

Alcohol.—Alcohol is very widely used as a germicide for the skin before needle punctures, etc. As a germicide it acts most efficiently at 70 per cent concentration. Absolute alcohol forms an albuminous coat around the bacteria and it does not penetrate and therefore does not kill the bacteria. Solutions weaker than 70 per cent are much feebler bactericides. Alcohol is a rather poor antiseptic but it has merit because it is an excellent cleaner of any oily substances on the skin which may contain infectious material.

Iodine.—The alcoholic tincture is the most popular form as a skin antiseptic. It produces a brown stain, does not sterilize the deeper layers of the skin and readily loses its antiseptic power when brought into contact with albumin. The B. P. preparation is 2.5 per cent and the U.S.P. preparation 7 per cent in 90 per cent ethyl alcohol. Sometimes methyl alcohol is used. These alcohols are oxidized on standing and form irritating products which are probably responsible for the iodine burns. These alcohols are also expensive. Isopropyl alcohol is equally solvent, does not oxidize to form irritating products and is much cheaper.[1] A 1.25 per cent iodine solution in 70 per cent isopropyl alcohol is recommended as a satisfactory substitute.

Mercurochrome 2 *Per Cent.*—Mercurochrome is only slightly lessened in antiseptic power in the presence of blood or urine. It is chiefly used as a genito-urinary antiseptic and it leaves an intense red stain that is difficult to remove. A 2 per cent solution in water is used for mucous surfaces and in 50 per cent alcohol and 10 per cent acetone as a skin antiseptic. (Mercurochrome 2 gm., distilled water

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[1] McDonald and Peck: Lancet, 1928, **215**, 443.

35 cc., 95 per cent alcohol 55 cc., acetone 10 cc.). The alcohol and acetone aid in penetrating the skin.

Up to the present it is not settled which is more efficient on the unbroken skin,[1] tincture of iodine or mercurochrome.

Acriflavine.—This is a most efficient skin antiseptic. After an application as outlined below, strips of skin cut from the margin of surgical incisions gave no growth on culture. (Acriflavine 1.0 gm. or 15 grains, alcohol 90 per cent 11 cc. or 3 drams. Acetone 2 cc. or 30 minims, water 7 cc. or 2 drams.[2]) First clean the skin well with soap and water, then alcohol, ether or any fat solvent until the sponges come away without discoloration. Prepare a fresh 5 per cent solution of acriflavine in a 50 per cent alcohol and 10 per cent acetone solution.

Hypochlorite Solutions.—This is an excellent wound antiseptic and should be freely used. The most convenient form is the chloramine tablets. They are put up in 4.6-grain size which makes a 1 per cent solution in an ounce of water. This is the optimum concentration. If large amounts are required the chloramine will be found expensive and Dakin's solution may be substituted.[3]

Practical Application of Germicides.—*For open wounds,* the hypochlorite solutions are best.

For sterilizing skin, tincture of iodine, acriflavine or mercurochrome in alcohol-acetone solutions. If the color is objectionable, as on the hands, use 1 in 1000 biniodine in 25 per cent alcohol or use a 1 per cent compound cresol or lysol. Alcohol 70 per cent for preparation of the skin prior to a hypodermic is satisfactory.

For instruments, compound liquor of cresol or lysol 1 to 2 per cent and rinse in sterile water.

For infectious discharges, excreta or contaminated clothing mix with an equal bulk of 5 per cent carbolic acid. A better preparation is a 2 per cent solution of liquor cresol compound (equal parts of cresol and soft soap) and leave standing one hour. Other solutions for this purpose are: Formalin 10 per cent. Add an amount equal to the volume to be disinfected and let stand one hour.

Chlorinated lime (fresh) 3 per cent is least expensive. Add an amount equal to the volume to be disinfected and let stand one hour.

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[1] Simons: Jour. Am. Med. Assn., 1928, **91**, 704. Reddish and Drake: Jour. Am. Med. Assn., 1928, **91**, 712.

[2] Tinker and Sutton: Skin Disinfection, Jour. Am. Med. Assn., 1927, **88**, 1560.

[3] *Dakin's Fluid.*—Dissolve 14 gm. of sodium carbonate in 1 liter of water and add 20 gm. of chlorinated lime that has not deteriorated. Shake the mixture. Allow to stand for one-half hour. Decant the supernatant fluid and add 4 gm. of boric acid to it. This solution is nearly neutral, and should contain 0.5 per cent of sodium hypochlorite. It will keep for a week in a stoppered container.

Care of the Hands.—It is as important for the laboratory worker as for the practising doctor to have socially clean hands at all times. Always wash the hands after doing any examination of a patient or of material which may be infected. This will go far in preventing the spread of infections.

The finger-nails should be short, neat and clean. Ordinary hand soap, a nail-brush and an abundance of running water, if possible, are the ideal cleansers. Soap is not only a mechanical cleanser but also has germicidal properties. A good lather with an ordinary soap and through washing of the hands will destroy any adhering diphtheria bacilli, streptococci, pneumococci, meningococci or gonococci, our most pathogenic bacteria.[1]

No corrosive chemical or infective material should come in contact with the skin. For handling material of this sort as well as for rough outside work the hands should be gloved. Absolute sterilization of the skin is impossible because antiseptics cannot reach the sweat ducts and so the sweat is always bacteria laden. Penetrating dye, antiseptics such as mercurochrome or acriflavine in alcohol-acetone solution are perhaps exceptions to this statement but their intense, indelible color renders their use on the hands impractical. Antiseptics incorporated in soaps are of questionable value and tend to give the user a false sense of security.

Bacteria from the sweat ducts are rarely virulent except when the hands have come in contact with virulent infections in wounds or discharges. After this the hands may harbor pathogenic microbes for weeks if they are not well cleansed. As an extra precaution the cleansing may be followed by an immersion in an antiseptic solution such as 1 in 1000 biniodide of mercury in 25 per cent alcohol to destroy any organisms that might remain in the crevices about the nails. Rubber gloves offer a smooth surface to disinfect and remove the hazard of infection from the sweat pores.

Frequent use of chemical antiseptics produces a roughened condition of the skin, which readily harbors organisms. If the skin of the hands be rough it should be specially treated. Usually a good hand lotion,[2] after washing will bring the skin back to natural

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[1] Walker: Jour. Inf. Dis., 1926, **38**, 127.

[2]

	Gm. or cc.	or	Drams.
Gum tragacanth	4		1
Alcohol	8		2
Boric acid	12		3
Glycerin	60		Ounces 2
Water to	500		Pint 1
Oil of bergamot	0.6		Minims 10

Mix the alcohol in the tragacanth. Heat the water and add the boric acid, glycerin and the tragacanth which has been previously mixed with the alcohol. The oil of bergamot may be added if desired.

smoothness. If the hands remain rough after a week of such treatment apply a bland fat or cold cream at night. Soap and water, especially the soap, is a great irritant to chapped hands so avoid soap but use as a substitute an abundance of hand lotion with a minimum of water and after rubbing this emulsion well into the skin, wipe it off on a clean towel. This lotion as a cleaner for the hands is nearly as efficient as soap.

Minor Injuries and Infections.—When an abrasion occurs on the skin pathogenic bacteria may gain entrance and set up an infection. One of the greatest aids in preventing infection is to induce bleeding for a few minutes either by holding the extremity down or applying a tourniquet. Bleeding, especially in puncture wounds, is the most effective means of washing out bacteria. Next treat the skin about the wound with a *non-irritating antiseptic* such as 2 per cent solution of mercurochrome. Strong solutions such as pure carbolic, lysol or formalin should never be used because they produce tissue necrosis which is a favorable nidus for bacteria to multiply. The wound should not be covered with adhesive or collodion but a natural scab be allowed to form, if the wound is small. A light porous dressing should be applied if the wound is large. This will allow the infective sweat to evaporate instead of flowing into the wound. The dressing should be changed daily. It should be covered by a glove only for short periods when necessary to protect it from infective material. If infection sets in, absolute rest and frequent hot arm baths should be instituted at once and a surgeon should be called to consider local drainage.

HYDROGEN-ION CONCENTRATION (pH).

pH is a measurement of the *intensity* of acid or alkaline activity.

The recently awakened interest in biochemical estimations has given much importance to the hydrogen-ion concentration of body fluids in health and disease.

The body has a capacity even more remarkable for maintaining its hydrogen-ion concentration than it has for maintaining its temperature. Slight changes in the reaction of the blood have a regulatory effect upon the respiratory center in the brain and a change in pH by lactic acid formation acts as a stimulus for muscular contraction. Neutralization of change in reaction is immediately accomplished through buffer substances such as hemoglobin acid and its salt, carbonic acid and bicarbonates, serum proteins and their salts, monobasic and dibasic phosphates. The excretion of acids and bases through the kidney is an important means of keeping the blood reaction at a constant point.

Special body cells in various organs are able to manufacture from the blood and body fluids secretions of different hydrogen-ion concentration which are best suited to the enzymes secreted in that organ. The saliva is usually slightly alkaline at which its enzymes work best.

The gastric contents is about a million times more acid than the blood and the salivary enzymes are inactive in this acidity while peptic enzymes are only able to exert their optimum action at a definite range of gastric acidity (pH 1.3 to 1.8). In cancer of the stomach hydrochloric acid is usually absent while in ulcer the acid concentration is usually excessive. Pancreatic enzymes are active only in the alkaline reactions of the intestine, which inactivate the gastric enzymes. For these reasons disturbance in pH of body fluid is important in that it may totally upset physiological function.

In bacteriology the pH of media is of the utmost importance. By setting the pH at a definite point it is frequently possible to isolate pure culture. For moulds the pH range is 1.5 to 8.5, for yeasts 2.8 to 8 and for bacteria the pH is 4 to 7.5. A few bacteria, especially the anerobes, have a very narrow pH range for growth.

pH 7 = neutrality.
pH 7.3 to 7.4 = reaction of human blood in health.
pH 7.1 to 7.2 = reaction of blood in acidosis.
pH 5 to 8 = reaction of normal urine.
pH 1 to 2 = gastric contents.
pH 1 = $\frac{1}{10}$ normal hydrochloric acid.
pH 13.2 = $\frac{1}{10}$ normal sodium hydroxide.

Hydrogen-ion estimation is the scientific method of recording the intensity of acid or alkali chemical activity in contrast to titration which is a measurement of the quantity of available acid or alkali. pH is the remains of a logarithmic symbol, which indicates the degree to which the hydrogen-ions separate out of a solution.

An acid is a solution that contains an excess of hydrogen ions (H^+) over hydroxyl ions (OH^-). An alkali contains fewer hydrogen ions than hydroxyl ions. A neutral solution like freshly distilled water contains an equal number of hydrogen and hydroxyl ions. It contains one ten-millionth gram of hydrogen ions and an equivalent amount of hydroxyl ions per liter and the pH is 7. The higher the hydrogen concentration the lower the pH figures, thus for 0.1 normal hydrochloric acid which contains 0.1 gm. of hydrogen per liter the hydrogen-ion concentration is 1.

Mineral acids such as hydrochloric form a high concentration of hydrogen ions even in very dilute solutions. This gives them great chemical activity. The organic acids do not form a high concentration of hydrogen ions even in concentrated solutions. The organic acids like acetic have only about one-hundredth the hydrogen-ion concentration that hydrochloric has.

Although this is true the total acidity of 10 cc. of tenth-normal acetic acid will neutralize 10 cc. of tenth-normal sodium hydroxide in the same manner as will 10 cc. of tenth-normal hydrochloric acid. This is due to the fact that as the hydrogen ions are neutralized, more separate out until they are all used up.

Indicators of Acid or Alkaline Reaction.—The oldest and commonest indicator is litmus. It is not a very sensitive one. It turns red when acid and blue when alkaline. Some indicators show their color change on the acid side of neutrality. Dimethyl-amido-azobenzol which is used in titrating for free hydrochloric acid in gastric contents, is an example of this. It changes at a pH of 3 to 4.

Phenolphthalein shows its color change slightly on the alkaline side of neutrality namely at a pH of 8 to 9.

A UNIVERSAL INDICATOR FOR HYDROGEN-ION CONCENTRATION.—By employing several indicators which show shades of color at various pH points it is possible to tell the approximate pH of a solution.

Reagent (*Dr. Bogen*).—Phenolphthalein, 0.1 gm.; methyl red, 0.2 gm.; dimethyl-amido-azobenzol, 0.3 gm.; bromthymol blue, 0.4 gm.; thymol blue, 0.5 gm. Dissolve these in 500 cc. of absolute alcohol. Add normal sodium hydroxide solution until the solution becomes a yellowish-green at a pH of 7.

The Test.—On a white porcelain plate add 1 drop of this universal indicator to 1 cc. of the solution to be tested and note the resulting color.

Red indicates about pH 2, very strongly acid.
Orange indicates about pH 4, strongly acid.
Yellow indicates about pH 6, weakly acid.
Green indicates about pH 8, weakly alkaline.
Blue indicates about pH 10, strongly alkaline.

Strips of filter paper dipped in this solution and allowed to dry give more information about pH and should replace the usual litmus paper.

STANDARD SOLUTIONS OF ACID AND ALKALI.

Standard solutions are of known strength and are generally referred to as normal or decinormal solutions.

A normal solution is one that contains 1 gm. of available hydrogen per liter. In hydrochloric acid there is 1 H atom, so the molecular weight obtained by totalling up the atomic weights of the elements ($H = 1$ and $Cl = 35.5$). 36.5 grams of the pure substance in 1 liter of water makes a normal solution. If the formula contains 2 H atoms, half the molecular weight is taken; the amount containing 1 gm. of hydrogen only. For sulphuric acid H_2SO_4, the molecular weight is $2 + 32 + 64 = 98$; but this contains 2 gm. of hydrogen so one-half the amount, 49 is taken. These normal acid solutions will exactly neutralize an equal amount of a normal alkali solution such as sodium hydroxide. The liter of normal sodium hydroxide contains 40 gm. of the pure substance which is made up by the atomic weights of sodium 23, oxygen 16 and hydrogen 1.

A decinormal solution is made by diluting 1 part of a normal solution with 9 parts of distilled water. They are used more than the normal solutions in medicine.

The neutralization point of an acid or alkali solution is determined by adding a few drops of an indicator and noting the change in color. Phenolphthalein is a suitable general purpose indicator. It is colorless in acid solution and red in alkali solution.

In actual practice it is not feasible to prepare directly normal solutions of hydrochloric acid or sodium hydroxide.

Hydrochloric acid is a heavy gas which readily dissolves in water making the preparation with which we are acquainted. Sodium hydroxide comes in sticks which absorb moisture from the air so that a definite amount of the chemical may vary in weight according to the amount of water it contains. Oxalic acid, even the commercial product is singularly free from other substances and its water content does not vary. Its formula is $H_2C_2O_4$ $2H_2O$ and the molecular weight would be $2+24+64+36=126$. The hydrogen in the water of crystallization does not affect the activity but as the chemical formula contains 2 H's, one-half the molecular weight must be taken to make a normal solution. As we shall require a decinormal solution only one-tenth is used, that is 6.3 gm. per liter of distilled water.

Preparation of Decinormal Sodium Hydroxide.—Dissolve 1.1 gm. of sodium hydroxide sticks in 10 cc. of distilled water and let stand for a few days. The sodium hydroxide will be on top in a clear solution while the sodium carbonate will remain as a turbid layer at the bottom. Pipet 0.6 cc. of the clear saturated solution and dilute to 1 liter with distilled water. This solution is slightly stronger than decinormal solution.

Weigh accurately 6.3 gm. of oxalic acid and dissolve in 1 liter of distilled water. Pipet exactly 10 cc. of the diluted sodium hydroxide solution into a clean vessel. Add 4 drops of phenolphthalein indicator and then add decinormal oxalic acid solution from a graduated 10-cc. pipet until the pink color just disappears. If it takes 13 cc. to do this, every 10 cc. of the sodium hydroxide must be diluted to 13 cc. with distilled water to make an exact decinormal solution. That is to 100 cc. of the sodium hydroxide 30 cc. of distilled water must be added to make it exactly decinormal. After this has been done the diluted solution should again be checked against the decinormal oxalic acid.

To Prepare Decinormal Hydrochloric Acid Solution.—Concentrated hydrochloric is about ten times normal. It should be diluted about 1 in 90 and then titrated with the decinormal sodium hydroxide. If 9.5 cc. of the acid added to 10 cc. cause the red of the phenolphthalein to disappear, to each 9.5 cc. add 0.5 cc. of distilled water to make exactly decinormal hydrochloric. Do a check test on the solution just diluted. Ten cubic centimeters of it should neutralize 10 cc. of the decinormal sodium hydroxide.

These solutions will keep well for years although the sodium hydroxide when stored in cheap glass containers forms a white precipitate which only very slightly impairs its strength. This can be prevented by coating the inside of the bottles with paraffin. Insert a small piece of paraffin in dry warm bottles and turn the bottles until the melted paraffin flows over all the inside. When the paraffin has hardened pour in the standard solution and cork. A glass stopper is best for the acid and a rubber one for alkali.

Except in large laboratories it is best to purchase a few ounces of the normal solutions ready prepared. From this the decinormal can be made by diluting 1 part with 9 of distilled water. An ampule of concentrated acid or alkali may be obtained which when dissolved in 1 liter of distilled water makes exactly a normal or decinormal solution.

Normal Saline Solution.—Normal saline solution used for hypodermic or intravenous treatment of blood depletion is quite different from the normal or hydrochloric acid. The normal of the physiological saline means that it has the same sodium chloride content as normal body fluids, namely 0.85 per cent.

PERCENTAGE SOLUTIONS.

Percentage solutions are easily made by the metric system: 0.1 per cent is 0.1 gm. or 100 mgm. in 100 cc.; 25 gm. to the liter makes a 2.5 per cent solution. Solutions are not so readily made with the Apothecaries' weight. For this reason the following rules are useful:

To Make 1 *Per Cent Solutions:*

5 grains (exactly 4.6) to the ounce makes a 1 per cent solution.

$2\frac{1}{2}$ drams to the U.S. quart which is 32 ounces makes a 1 per cent solution.

$3\frac{1}{4}$ drams to the Imperial quart which is 40 ounces makes a 1 per cent solution.

1 minim of a 1 per cent solution contain $\frac{1}{100}$ grain.

To Obtain a Dilute Solution from a More Concentrated One:

1. *Percentage Dilutions.—Example.*—To obtain 12 cc. of a 24 per cent solution from an 80 per cent solution take of the latter $\frac{12 \times 24}{80} = 3.6$ cc. and make up to 12 cc. with the diluting fluid.

2. *Fractional Dilution.—Example.*—To obtain 60 minims of a $\frac{1}{100}$ dilution from a $\frac{1}{10}$ dilution take of the latter $\frac{60 \times 10}{100} = 6$ minims and make up to 60 minims with the diluting fluid.

3. *To Strengthen a Solution by the Addition of a Solid.*—To make 100 cc. of 25 per cent solution from a 20 per cent solution, take nearly the 100 cc., that is 90 cc. of the 20 per cent solution. It contains $\frac{90}{100}$ of $20 = 18$ gm. Add 7 gm. of the pure substance to make 25 gm and add the diluting fluid to 100 cc.

4. *The Preparation of a Solution One of Which is Stronger and the Other Weaker Than the Required One:*

Alligation may be employed to obtain a desired percentage solution from several solutions already prepared, some of which must have a greater and some a lesser percentage than that of the required solution.

Write the percentage strength of the prepared solutions in a column and the desired percentage to the left. Bracket the prepared percentages in pairs, one less than the desired solution with one greater. Take the difference between the desired percentage and the number representing the percentage strength to which it is linked. These different figures represent the relative quantities of the solutions used to make the desired percentage. If the number of the already prepared solutions are uneven two or more of the weaker solutions may be bracketed with one of the stronger or *vice versa.*

Example.—To make a 45 per cent solution by mixing a 15 per cent and a 70 per cent solution.

$$\begin{array}{lll} & 70 & 30 \\ 45 & & \\ & 15 & 25 \end{array}$$

Of the 70 per cent solution take 30 parts and of the 15 per cent solution, 25 parts. If a liter is required $\frac{30}{25+30}$ of 1000 cc. = 545.4 cc. of the 70 per cent solution and the remainder of the 15 per cent. Where there are three solutions of different strengths to mix to make a desired strength.

90 per cent, 65 per cent, 30 per cent are the already prepared ones and a 50 per cent is to be made by mixing them.

$$\begin{array}{llll} & 90 & 20 & \\ 50 & 65 & 20 & \\ & 30 & 15 & + 40 \end{array}$$

Of the 90 per cent solution take 20 parts, of the 65 per cent solution take 20 parts and of the 30 per cent solution take 55 parts.

COLORIMETERS.

Many of the important tests recently employed in medicine make use of the colorimeter. Hemoglobin, blood sugars and the red dye test of kidney function are prominent examples. The use of the colorimeter in a test has some objections. Some have difficulty in matching certain colors and at times the solutions have not the same tint. On the other hand this procedure has shortened many tests and it will probably be used more frequently in new ones which may come out from time to time. For these reasons it is important to know the principle on which colorimeters work.

To illustrate the principle, the method of estimating the sugar in the blood may be taken as an example. After the albumin is removed an excess of chemicals is added, which with sugar, will make a blue color. The intensity of the color will depend upon the amount of sugar present. If there is much, the solution will be very blue. A test on standard 0.1 per cent solution of glucose is done simultaneously with one on blood and the intensities of the solutions compared. If the depth of color in the solution containing the blood freed from protein be twice that of standard then the blood contained 0.2 per cent of sugar.

To make this estimation a colorimeter is necessary and the various makes divide themselves into two groups:

1. (*a*) *Cup and Plunger Type,* in which we match the intensity of colors by varying the depths of the columns of fluids through which we look. This type is the most accurate and is quite expensive. The Buerker type manufactured by Leitz & Co., costs $120 and the Klett type costs $60.

(*b*) *Wedge Type,* not quite so accurate as the cup and plunger type but convenient for clinical use because no calculation is required. They cost about $50.

2. *The Dilution Type.*—Two test-tubes of equal bore in which the matching is accomplished by diluting one solution, the depth of vision through the tube remaining constant. They are very inexpensive but have an error of 2 to 5 per cent.

1. (*a*) **Cup and Plunger Type.**—With this type one looks down into an eyepiece which is divided into two equal parts. Each half by a series of mirrors directs the vision down through a glass plunger into a glass cup. One of these cups contains the standard colored solution and the other the same substance but of unknown strength. The light is reflected up through these solutions from a mirror below. These cups may be moved up and down to alter the depth of the solution and this is accurately registered on a scale at the side of the colorimeter. After pouring the same solution into each cup, if the depth of one be set at 20 mm. and the other moved up and down until the colors appear equal, the depth of the other will also be 20 mm.; that is two columns of equal depth of a solution of equal strength show an equal intensity in color. Likewise if one solution, A, contains twice as much of a colored substance as solution B it will take twice the depth in the column of B to give a color intensity equal to A. With a solution C containing 15 mgm. per 100 cc. of a colored substance as a standard in one cup at a depth of 20 mm. and matched with a solution D containing the same substance but unknown quantity in the other cup at a depth of 25 mm. the D solution contains $\frac{20}{25}$ of 15 = 12 mgm. per 100 cc.

Technical Details.—The reflection mirror at the bottom should be clean and placed so that the cups will be equally illuminated. If one side through constant use becomes brighter the darker side should be scrubbed with a grit soap such as "Bon Ami" powder to bring the whole surface into uniformity. The cups should be thoroughly clean and moved up so that when the plunger is at the bottom of them the scale at the back should register exactly 0. If it registers half a millimeter, that amount must be added to the final reading. On the best makes the scale may be adjusted so that it registers 0 when the plunger is at the bottom of the cup.

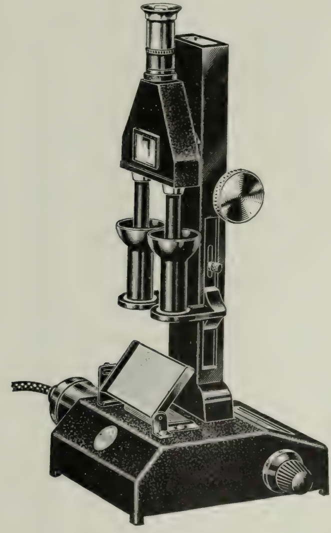

Fig. 106.—Colorimeter.

To Use.—Fill both cups with the standard and adjust the left hand one so that the scale reading is exactly 20 mm. If 20 mm. depth gives a color that is too deep for matching use 15 as a standard. Then move the right hand cup up and down until the color is exactly the same as that of the other. This is best accomplished by a series of quick glances rather than a prolonged look. Repeat several times and take the average of the readings. The right hand cup should be set at this average because it gives a color which is equal to 20 in the left hand cup. The standard in the left hand cup is discarded and the cup and plunger rinsed with the unknown solution. After the cup is nearly filled with the unknown and moved up and down until colors on both sides are equal, three readings are then made and the average taken. The results are computed by using the following formula:

$x = \frac{20}{y}$
20 = scale reading in cup containing known solution.
y = scale reading in cup containing unknown.
x = the concentration that the unknown is of the known standard.

Example.—y, the scale reading in the cup containing the unknown is 12 mm. Unknown is $\frac{20}{12}$ or $1\frac{2}{3}$ of standard. The standard contains 100 mgm. of glucose per 100 cc. (0.1 per cent). Therefore the unknown is $1\frac{2}{3} \times 100 = 166$ mgm. per 100 cc. (The standard is composed of a number of chemicals in excess which with glucose make a blue color so that the intensity of blue will depend on the amount of glucose present.)

(*b*) *Wedge Type of Colorimeter.*—With this instrument the standard solution is placed in a wedge-shaped glass so that it is deep in color at the top and faint in color at the bottom. The unknown solution is placed in a small cup at the side of the wedge. The wedge is moved up until it matches the unknown exactly and the percentage that the unknown is of the standard is read from a scale at the side of the wedge. With this type, if the standards are solutions, one must guard against evaporation or deterioration. The cup for the unknown should be kept very clean.

2. **The Dilution Type of Colorimeters.**—These depend on the principle that solutions containing the same percentage of a colored substance give an equal color when viewed through the sides of equal sized test-tubes.

Choose 2 test-tubes of equal size. Long narrow ones are best. As it is very necessary to have the tubes of exactly the same diameter put an equal amount of water into each test-tube. If they are of equal internal diameter the fluid height will be the same in both. Put exactly 5 cc. of the denser solution into one tube and half fill the other tube with the less dense solution. Hold the tubes to the light with a thin sheet of white paper behind them and using a pipet add diluting fluid, which is usually water, to the denser fluid. Keep an accurate record of the amount of diluent required to make them equal in color. After each addition of diluent invert the tube so the color will be uniform.

When the solution being diluted approaches the other in intensity the diluent should be added very carefully, a little at a time until the colors are equal. If too much diluent is added no estimation can be made and the test should be repeated.

When the standard has to be diluted divide its strength by its cc. content after dilution and multiply by 5 to obtain the strength of the unknown. When the unknown has to be diluted multiply the strength of the standard by the content of the unknown after dilution and divide by 5.

Examples.—(*a*) With known solution containing 3 per cent of dye and denser than the unknown.

Original amount of known in test-tube 5 cc.
Final amount of known to make it equal to unknown 12 cc.
Unknown is 3×5 divided by $12 = 1.25$ per cent.

(*b*) With known solution containing 3 per cent but less dense than unknown which will have to be diluted to make them equal.

Original amount of unknown in test 5 cc.
Final amount of unknown in test-tube 18 cc. to make both equal.
Unknown is 3×18 divided by $5 = 30$ per cent.

To facilitate the measurements Victor Meyers has devised two long slender test-tubes of equal bore and graduated to show at a glance the amount of their contents. These cost only $3.20 and are very satisfactory. A stand which contains a frosted glass and a diluting pipet make the set complete at a cost of $12, but these add little or nothing to the working value of the apparatus.

For the phenol red dye test of kidney function we have used in classwork, the plain test-tubes or 100-cc. cylinder graduates of equal bore so the 100-cc. marks would be of equal height. The readings done by students for the first time were never more than 5 points out and frequently only 2 to 3 away from those made with the best types of cup and plunger colorimeters.

An additional type might be mentioned in which there are a number of standard ampules showing varying degrees of a color. The unknown is placed in an open ampule and matched with the standards. The standards bear the percentage readings they represent. Common examples of this type are the Dunning colorimeter for the phenol red dye test of kidney function, the standards of the Marriott apparatus for determining the carbon dioxide in alveolar air in cases of acidosis and the Tallqvist scale for the determination of hemoglobin.

To estimate the percentage of a dye such as bromsulphalein in blood serum, the alkalinized serum must be compared with an equal sized tube containing the watery solution of the standard dye and a tube of normal serum in front of it.

TEMPERATURE.

The Centigrade system is employed in scientific work for recording temperatures. The freezing-point of the Centigrade is 0 and its boiling-point 100 degrees. The every-day Fahrenheit system, which has for its freezing-point 32 and for its boiling-point 212 degrees, is used for recording temperatures in medical science occasionally. It is necessary to know how to convert temperature readings from one to the other.

To convert a temperature in Centigrade to its Fahrenheit equivalent multiply by $\frac{9}{5}$ *and add to the result* 32.

To convert a temperature recorded in the Fahrenheit to its Centigrade equivalent first subtract 32, *then multiply by* $\frac{5}{9}$.

The following chart shows the equivalent Centigrade and Fahrenheit temperatures and common reactions that occur at various levels:

Cent. *Fahr.*

125 257—Produced by 20 pounds of steam per square inch in autoclave kills *all microbes and spores* in fifteen minutes. Dry heat at this temperature will kill microbes but not spores in ten minutes.

100 212—Moist heat kills *all microbes in one minute and the pathogenic spores such as tetanus in twenty minutes.* It does not kill the non-pathogenic spores such as Bacillus subtilis. Twenty minutes on three successive days will accomplish this.

90 194—Copper reduction tests for sugar occur at this temperature.

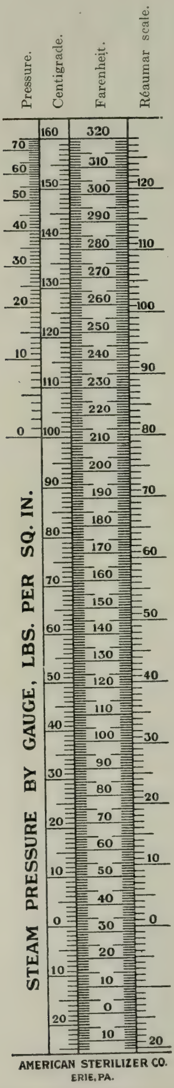

FIG. 107.—Cut of equivalent Fahrenheit and Centigrade temperatures.

75 167—Albumin coagulates.
65 149—Moist heat kills ordinary forms of pathogenic microorganisms such as staphylococci and streptococci in ten minutes. It does not destroy pathogenic spores like tetanus, however.
55 131—Destroys complement in serum in fifteen minutes.
45 113—And above causes pain on the immersed hand.
40 104—Feels warm.
37 98.6—Body heat. Best for growing microbes.
26 75—Feels cool to hand.
20 to 21, 68 to 70—Room temperature.
10 50—Bacterial growth ceases but will begin again when temperature is suitable.
10 to 4.50 to 39—Is best for preserving serums and vaccines.
0 32—Freezing.

WEIGHTS AND MEASURES.

The Metric system with its decimals is very practical for scientific work. Pounds, ounces and grains are used for the most part in commerce and pharmacy. For measuring length the metric system is best but so many think and record in inches that in clinical laboratory work one must know both systems and convert readily from one to the other.

The milligram is used for weighing very small amounts. A concrete idea of its weight may be had by realizing that a ½-inch square of paper such as this book is made of, weighs 15 mgm. This is a normal urea nitrogen content in 100 cc. of blood. The 100 cc. represents a measure of nearly 4 ounces. Most of the estimations in blood chemistry are reported in milligrams per 100 cc. of blood.

The gram is the unit of weight most frequently used in laboratory work. It is equivalent to 1000 mgm. and a concrete idea of its weight may be had by remembering that a dime weighs about 2⅓ gm.

The kilogram in the metric system is used where we use the pound in the old system. It is equal to 2⅕ pounds.

Metric Weights.

1000 milligrams (mgm.) = 1 gram (gm.) = 15⅖ grains (gr.)
1000 grams (gm.) = 1 kilogram (kgm.) = 2⅕ pounds (lbs.).

Apothecaries' Weight.

60 grains (gr.) = 1 dram (ʒ) = 3.9 grams (gm.).
8 drams (ʒ) = 1 ounce (℥) = 31.1 grams (gm.).
12 ounces (℥) = 1 pound (lb.) = 373.2 gram.

Metric Lengths. *Equivalent in inches and feet.*

1000 microns (1) (μ) = 1 millimeter = $\frac{1}{25}$ inch.
10 millimeters (mm.) = 1 centimeter = $\frac{2}{5}$ inch.
100 centimeters (cm.) = 1 meter = $39\frac{2}{5}$ inches.
1000 meters (M.) = 1 kilometer (Km.) = $\frac{5}{8}$ mile.

1. **Microscopic Measurements.**—*Equipment.*—The Metz Echelon Micrometer Eyepiece sold by E. Leitz and containing simplified micron divisions is most convenient for routine work.

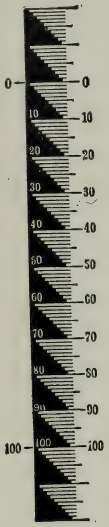

FIG. 108.—View through the microscope with a Metz Echelon Micrometer Eyepiece. (Ernst Leitz, Wetzler.) With the oil-immersion lens, one space is one micron

A microscope and hemocytometer are also necessary. To find out what each division on the micrometer scale represents with the various objectives, place the central ruled part of the hemocytometer that is used for counting red cells under the microscope. The squares are in blocks of 16 and each small square is $\frac{1}{20}$ mm. or 50 microns in width.

For Measuring Large Parasites Use the Low-power Lens.—With the low-power objective 5 divisions will extend across the 50 micron square, therefore each division on the micrometer eyepiece will be 10 microns.

For Measuring Smaller Objects Such as Ova Use the High-power Lens.—With the high dry objective lens if 25 divisions will cross the 50 micron square each division on the micrometer scale will be 2 microns. Changing the tube length will adjust this exactly.

To determine the size of very small objects such as blood cells and bacteria use the oil-immersion lens. Place the hemocytometer without its cover-glass under the high dry lens and when the fine central cross lines are in focus, turn in the oil-immersion lens. Do not use any oil as it obscures the lines completely. Lowering of the Abbe condenser will make the lines more distinct. If 50 of the fine divisions on the micrometer scale will cross a small square each division represents 1 micron. Changing the draw tube length of the microscope until 50 divisions on the micrometer scale cross the 50 micron square on the counting chamber will make each division exactly equal to a micron. The hemocytometer may now be removed and as long as the microscope tube remains drawn out to the proper length and the oil-immersion lens is used, each division will represent a micron.

The Value of Cell Measurements.—Cell measurements are of value in differentiating anemias. The range of diameter in normal red cells is 6 to 9 microns. In pernicious anemia the cells may range from 4 to 12 and those above normal size, the megalocytes are strong evidence in favor of that disease. In secondary anemia the red cells may be from 3 to 9 microns.

Cell measurements are of value in classifying the white blood cells. If in doing differential counts, one wishes to distinguish between small and large lymphocytes, 9 microns may be taken as an arbitrary point. The polymorphs will be from 12 to 20 microns with a slightly irregular cell and nuclear outline, may be called a large mononuclear or monocyte.

Metric Fluid Measure.

1000 cubic millimeters (cu.mm.) = 1 cubic centimeter (cc.).
or milliliter (mil. or ml.) = 16 minims.
1000 cubic centimeters (cc.) = 1 liter (L.) = 33.8 fluidounces approximately 1 U.S. quart or $1\frac{7}{10}$ Imperial pints (exactly 1 quart and 7 drams).

A cubic millimeter may be visualized by remembering that it is about equal in size to the head of a common pin. It is the volume unit in which blood cell counts are recorded.

The cubic centimeter is the most frequently used fluid measurement in the clinical laboratory. About 4 fill a teaspoon.

Fluid Measure.

60 minims (m. = 1 fluid dram = 3.7 cc.
8 fluid drams (fl ʒ) = 1 fluid ounce = 30 cc. (exactly 29.57 cc.).[1] (U.S.)
16 ounces = 1 fluid pound (lb) = 1 pint (U.S.) = 473 cc.
20 ounces = 1 pint (Imperial) = 568 cc.
2 pints = 1 quart.
4 quarts = 1 gallon. Weight (U.S.) 8.33 pounds, Imperial 10 pounds distilled water.

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[1] A fluidounce in Apothecaries' or troy weight is 31.10 gm. and in Avoirdupois Imperial weight is 28.35 gm.

The minim is not equal to a drop. The drop is large if the point from which it falls is blunt. A drop of water poured from the mouth of a bottle is equal to $1\frac{1}{4}$ minims, of 90 per cent alcohol it is $\frac{2}{3}$ of a minim. A drop of water from an ordinary medicine dropper is about $\frac{4}{5}$ of a minim but alcohol from the same dropper is $\frac{1}{4}$ of a minim. Many medicine droppers are made with a special bulb end so that the drop of a certain alcoholic solution such as a tincture is equal to $\frac{1}{2}$ of a minim. It is best to use a graduated minim measure.

The fluid pound which is made up of 16 fluidounces is the measure used frequently in selling chemical solutions such as acids, tinctures, etc., irrespective of the actual weight of the solution. Ether and chloroform are sold by actual weight.

Length Equivalents.

1 millimeter (mm.) = $\frac{1}{25}$ of an inch.
1 centimeter (cm.) = $\frac{2}{5}$ of an inch.
1 inch = 25.4 millimeters or 2.5 centimeters.

Weight Equivalents.

1 milligram (mgm.) = 0.015 grains, or approximately $\frac{1}{65}$ grain.
1 gram (gm.) = 15.4 grains.
1 kilogram (kgm.) = $2\frac{1}{5}$ pounds.
1 pound Avoirdupois = 453.5 grams.
1 ounce Avoirdupois = 28.3 grams.
1 ounce Apothecaries' = 31.1 grams.
1 dram Apothecaries' = 3.9 grams.
1 grain Apothecaries' = 65 milligrams (mgms.).

Fluid Measure Equivalents.

1 cubic centimeter (cc.) or 1 mil. = 16 minims.
1 liter (L.) = 33.8 fl. oz. or 2.1 pints (U. S.).
1 fl. ounce (Apothecaries') = 30 cubic centimeters.
1 fl. ounce (Avoirdupois) = 28.4 cc.
1 pint (U. S.) = 473 cc.
1 pint (Imperial) = 568 cc.
1 gallon (U. S.) = 3.785 liters.
1 gallon (Imperial) = 4.546 liters.

Factors for the Conversion of Measures in One System to the Other.

To Convert into Metric Weight.

Grains Apothecaries' or Troy to milligrams multiply by 64.8.
Grains Apothecaries' or Troy to grams multiply by 0.0648.
Drams Apothecaries' or Troy to grams multiply by 3.88.
Ounces Apothecaries' or Troy to grams multiply by 31.1.

Ounces Avoirdupois to grams multiply by 28.36.
Pounds Avoirdupois to Kilograms multiply by 0.454.
Pounds Apothecaries' or Troy to kilograms multiply by 0.373.

To Convert from the Metric System.

Milligrams to grains Apothecaries' or Troy multiply by 0.0154.
Grams to grains Apothecaries' or Troy multiply by 15.432.
Grams to drams Apothecaries' or Troy multiply by 0.257.
Grams to ounces Apothecaries' or Troy multiply by 0.0311.
Grams to ounces Avoirdupois multiply by 0.0353.
Kilograms to pounds Avoirdupois multiply by 2.2046.
Kilograms to pounds Apothecaries' or Troy multiply by 3.732
Grams per 100 cc. of fluid to grains per fluid ounce multiply by 4.6.
Grains per fluid ounce to grams per 100 cc. of fluid multiply by 0.22.

To Convert into the Metric System.

Hundredths of an inch to microns multiply by 250.
Inches to millimeters multiply by 25.
Inches to centimeters multiply by 2.5.
Inches to meters multiply by 0.0254.
Feet to meters multiply by 0.3048.
Miles to Kilometers multiply by 1.61.

To Convert Measure of Length from the Metric System.

Microns to hundredths of an inch divide by 250 or multiply by 0.004.
Millimeters to inches divide by 25 or multiply by 0.04.
Centimeters to inches divide by 2.5 or multiply by 0.4.
Meters to inches multiply by 39.37.
Meters to feet multiply by 3.28.
Kilometers to miles divide by 1.61 or multiply by 0.62.

To Convert into the Metric Fluid Measure.

Minims to cubic millimeters (cu.mm.) multiply by 63.
Minims to cubic centimeters multiply by 0.06.
Fluid drams Apothecaries' to cubic centimeters multiply by 3.7.
Fluid ounces Apothecaries' to cubic centimeters multiply by 30.
Fluid ounces Avoirdupois to cubic centimeters multiply by 28.4.
U. S. pints to liters multiply by 0.473.
Imperial pints to liters multiply by 0.568.
U. S. gallons to liters multiply by 3.784.
Imperial gallons to liters multiply by 4.544.

To Convert Fluid Measure from the Metric System.

Cubic millimeters (cu.mm.) to minims divide by 63 or multiply by 0.016.

Cubic centimeters (cc.) to minims multiply by 16.

Cubic centimeters to fluid drams Apothecaries' multiply by 0.27.

Cubic centimeters to fluid ounces Apothecaries' divide by 30 or multiply by .033.

Cubic centimeters to fluid ounces Avoirdupois divide by 28.4 or multiply by .035.

Liters to pints U. S. multiply by 2.1.

Liters to pints Imperial multiply by 1.76.

Liters to U. S. gallons multiply by 0.26.

Liters to Imperial gallons multiply by 0.22.

THE TEACHING OF CLINICAL LABORATORY METHODS.

The period of class study for laboratory methods should not be shorter than two or three hours. Twenty to thirty such periods will cover the work necessary in clinical laboratory methods. The instructor should outline for the term the one or two reactions he wishes to teach each period. All equipment and material required should be laid out before the class begins. A class should open by the instructor giving a practical demonstration of the procedure to be practised. This may occupy ten to fifteen minutes, after which time the students go to practice this work themselves. It is impossible for them to remember all the steps in a procedure they have seen for the first time so they should work in groups of two's, one reading the directions from the text while the other carries out the detail step by step. After this the student who has read should carry out the test while his partner reads the directions. If several demonstrators are present each one should be allotted certain students to prevent overlapping each other's work.

The following are the simple routine tests given in order of their utility: urine analyses, chemical and microscopic; hemoglobin estimation and blood counts; pathogenic organisms in exudates and sputum; analysis of stomach contents; examination of feces for blood, parasites and ova; cerebrospinal fluid examination; blood grouping and matching.

It is necessary to supplement this classwork. Probably the two most important examinations the student makes is on micro-urine analysis and blood counts. He cannot examine too many micro-urines and whenever possible a urinary sediment should be present each period throughout the term for him to examine in addition to his usual work. He should also spend a period of a week in the hospital ward and out-patient's laboratory, assisting in all the routine examinations as they come and examining microscopically all the urinary sediments. By this means he should become thoroughly acquainted with the casts, pus and blood in their various amount and stages of degeneration as well as other unimportant material that is occasionally present such as cotton fibers, amorphous crystalline salts, etc.

When a patient comes for full blood count the technician and student should fill pipets from the same incision and compare results. If the patient is not available the student may acquire skill equally well at blood counting by doing on his own blood a daily full blood count, including red cell count and hemoglobin determination, a total and differential leucocyte count.

He should be methodical in his procedure and lay out all equipment in readiness. Then take specimens of blood in the following order, wiping away between each to secure fresh blood for the next, hemoglobin, white cell count, red cell count, two blood smears.

The specimens requiring the largest amounts of blood are taken first. Most time is saved by doing some examinations while other reactions are taking place. Allow the blood to stand in contact with the acid for the hemoglobin estimation. Examine the unstained smears and then stain them. While the staining is taking place count the white and red cells. After this do the differential count and last dilute the hemoglobin tube with water until it matches the standard. By following this procedure there is no waiting and the hemoglobin and staining reactions are given ample time.

Not only will the student acquire practice in doing blood examinations but he will be shown very forcibly any large variation due to a mistake in technique and a small variation which is normal from day to day. No student should pass the practical examination without showing that he has a thorough grasp of microscopic urine analysis, blood counts, smears of blood, pus and bacteria. Next in importance is the correct procedure in collection of all specimens for examination by laboratory methods. After this he may familiarize himself with the more extensive laboratory studies in important diseases.

The following specimens mounted on slides will make a helpful nucleus for a student's collection. The films should all be mounted under a cover-glass to preserve them from dust or abrasion, and be neatly labelled. Additional specimens should be added from any field of medicine that holds a special interest for the student.

Blood.

Normal film.
Leucocytosis—about 20,000 per cu.mm.
Reticulocytes.
Stippling.
Malarial parasite.
Marked hemorrhagic anemia.
Slight hemorrhagic anemia.
Addison's anemia.
Lymphatic leukemia.
Myelogenous leukemia.

Cerebrospinal Fluid.

Lymphocytes (methylene blue).

Pus and meningococci	Methylene blue and Gram's.
Pus and pneumococci	
Pus and streptococci	

Smears of Exudates.

Acute gonorrhea—methylene blue
Subacute gonorrhea—methylene blue and Gram's.
Chronic gonorrhea—methylene blue and Gram's.
Uterine cervical smear—non-gonorrheal—methylene blue and Gram's.
Pus from a boil.
Tubercle bacilli in sputum.
Diphtheria culture.
Smear of Vincent's organisms.
Smear for chancre containing Spirochæta pallida—Cerquir's stain.
Ringworm on hair—modified Gram's stain.
Oppler-Boas bacilli—Gram's stain.
Pinworm.
Parasitic ova of roundworm and fish tapeworm from stool.

IMPORTANT RULES ON THE USE OF A MICROSCOPE.

Bright daylight is best, but avoid direct sunlight. Any electric lamp with a frosted bulb is satisfactory.

Always examine every specimen with low power first. It shows twenty times the area of the high dry lens.

Examine stained specimens with diaphragm open and unstained specimens with diaphragm closed.

Bring the objective lens nearer the slide than the proper focus, then with the eye at the eyepiece, focus up until the object is clearly seen.

If the object be not clearly seen, examine and clean the objective lens, adjust the diaphragm and mirror to obtain good lighting. Turn the eyepiece to detect any particles on its lenses and make sure the slide is right side up.

When the examination is finished, turn back the low-power objective in focus, wipe off the oil and cover with a lintless cloth.

RECORD OF LABORATORY EXAMINATIONS.

(For Use Reproduce as Charts 11 Inches x 8½ Inches.)

Name.. Date........................

Urine analysis.

Date										
N. = Normal										
A. = Abnormal										
Type of sample										
Amount										
Color (D—dark; L—light)										
Turbidity										
Reaction										
Specific gravity										
Albumin (per cent)										
Sugar (per cent)										
Ketones { Sod. nitroprusside										
Ketones { Ferric chloride										
Bile										
Centrifuged sediment										
Pus cells (per H.P. field)										
Red blood cells (per H.P. field)										
Hyaline casts (on slide)										
Granular casts (on slide)										
Cellular casts (on slide)										

Test-meal.

Date			
Amt. (oz.)			
Appearance			
Free HCl			

Stool.

Date			
Blood (occult)			
Parasites			
Ova			

Sputum.

Date			
Amt. (oz.)			
Character			
Cells			
Bacteria			

Hematological examination.

Date						
Hemoglobin (per cent)						
Red blood cells (million per cu.mm.)						
Color index						
Icterus index						
Blood film						
Achromia						
Poikilocytosis						
Anisocytosis						
Microcytes						
Macrocytes						
Normoblasts						
Megaloblasts						
Polychromasia						
Stippling						
Reticulocytes (per cent)						
White blood cells (thousand per cu.mm.)						
Number of cells counted in differential						
Lymphocytes (per cent)						
Monocytes (per cent)						
Eosinophils (per cent)						
Neutrophils (solid nucleus) (young)						
Polymorphonuclears (segmented nucleus) (mature)						
Undetermined (per cent)						
Platelets (thousands per cu.mm.)						

Wassermann.

Date	Reaction

Basal metabolism.

Date	Reading

Blood chemistry (fasting blood, mgm. per 100 cc.).

Date			
Sugar			
Urea N.			
Creatinine			

Spinal fluid	Date

Blood culture	Date

Widal	Date

Miscellaneous tests	Give dates

0 = Absent. v = Present. 1 = Slight. 2 = Moderate. 3 = Extreme.

To use the following outlines of tests to advantage they should be typed on sheets of paper 8 by 11 inches, mounted and placed on the wall for ready reference, opposite the bench where the various tests are performed.

Mounting is best done by placing the sheets between cardboard and cellophane or X-ray film washed transparent, then bound around by adhesive strip. To render old X-ray films transparent, wash off the emulsion with hot water and a soft cloth. Hang up to dry.

LABORATORY MEDICINE—NICHOLSON.

OUTLINE OF HEMOGLOBIN ESTIMATION. (SAHLI'S METHOD.)

Place 20 cu.mm. of blood from the pipet into the graduated tube which contains tenth-normal hydrochloric acid up to the "10" mark, and shake to mix. Dilute with tenth-normal hydrochloric acid until the solution matches the standard. The figure opposite the top of the column of solution denotes the percentage of hemoglobin. Normal—75 to 110 per cent.

EXAMINATION OF AN UNSTAINED BLOOD SMEAR.

Place a small drop of blood near the end of one slide. Spread it out with the edge of a cover-glass or other spreader, by making the blood follow the moving spreader.

Examine the unstained film unmounted under the high dry lens and subdued light, for irregularity in shape and size of the red cells. The relative frequency of white cells and the platelets may be clearly made out.

BLOOD COUNTS—GENERAL.

Obtain a large drop of blood. After filling the pipet shake it well. Discard the first drop. The diluted blood must not overflow the counting chamber platform. Immediately after use, clean the chamber and pipets. Blow out any cells remaining in the bulb. Fill twice and empty (1) with water, (2) with acetone, (3) draw air through to dry.

LEUCOCYTE COUNT.

1. Draw blood up to 0.5. Dilute with 2 per cent acetic to "11" mark.

2. Use lower power and closed diaphragm. Count all cells in 4 corner squares, each made of 16 small squares and multiply the total by 50. Normal 4000 to 12,000 per cu.mm.

RED CELL COUNT.

1. Draw blood to 0.5. Dilute with Hayem's fluid to 101.

2. Use the high dry lens. Count 5 large squares bounded by double lines, each including 16 small squares. Add 4 zeros to total for number of cells per cubic millimeter. Normal 4 to 6 million.

LABORATORY MEDICINE—NICHOLSON.

OUTLINE OF TECHNIQUE FOR THE BLOOD SMEAR.

Routine stain of eosin and hematoxylin for all features listed below except 5, 6 and 7 which are infrequently required. Alcoholic eosin one to two minutes. Wash. Hematoxylin two to five minutes. Wash.

Special stains of Wright and Leishman for all features listed below. Stain with 8 drops for one minute. Add an equal amount of distilled water for three to five minutes. Flood off with distilled water. Mount.

For a general examination use the high dry lens WITH THE STAINED FILM MOUNTED. For minute detail use the oil-immersion lens.

EXAMINE FOR THE FOLLOWING FEATURES. FREQUENT AND IMPORTANT FINDINGS.—1. Achromia.—An unusual lack of color in the center of some or all red cells.

2. Poikilocytosis.—A deviation from the circular outline, not artefact.

3. Anisocytosis.—An irregularity in the size of the cells (a) Normocytes, red cells of normal size. (b) Microcytes, red cells smaller than normal, (c) Macrocytes, red cells larger than normal.

Infrequent Findings. 4. Nucleated Red Cells.—(a) Normoblasts. A cell similar in size and cytoplasm to the red cell, but having a small blue nucleus.

(b) Microblast.—A nucleated red cell, smaller than a normoblast.

(c) Megaloblast.—A large red cell with pale cytoplasm and a nucleus.

5. Basophilic Stippling.—Minute black dots in occasional red cells.

6. Polychromatophilia.—An irregularity in staining of the red cells.

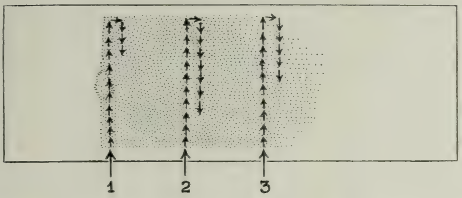
7. Malarial Parasites.—Appear in the cell as blue ring forms, crescents or bands, small or large and with red or black dots in them.

Important Observations Other than Red Cells.—8. Blood Platelets—occur in clumps. Note approximate change in their 1 in 25 ratio to red cells.

9. White Cells.—More occur at the end and margin of the smear than in the center; note the approximate change in their 1 in 1000 ratio to red cells, if increased whether lymphocytes or polymorphonuclears.

10. Differential White Cell Count.

DIFFERENTIAL WHITE CELL COUNT.



For a differential count move the slide to count 100 cells across the film at 1. Then make a similar count at 2 and 3 and take the average.

The Count.—Count about 100 white cells at 1, 2 and 3. Record them by the tally system and calculate the percentage of each variety.

	NORMAL PER CENT.	TALLY.	PER CENT.
1. Lymphocytes . . .	20 to 30	~~1111~~	
2. Monocytes	5 to 10	11	
3. Eosinophils, polymorphonuclear . . .	2 to 5	1	
4. Neutrophil polymorphonuclear .	60 to 75	~~1111~~ ~~1111~~	
(unsegmented 5 to 10)			
(segmented 55 to 70)			
5. Myelocytes	0		

6. Undetermined types (usually number 2, 5 or 6).

The basophil and myeloblast are omitted from the list because they are rare cells and of no diagnostic importance.

If 300 cells are counted the error is less than 3 per cent.

If only 100 are counted the error is over 10 per cent. (Beacom.)

LABORATORY MEDICINE—NICHOLSON.

OUTLINE OF EXAMINATION OF PUS SMEARS.

Make two thin smears in every case. Dry them at once. Fix by moderate heat before staining.

Slide 1. Stain with methylene blue for one minute. Wash, blot and dry. This stain gives the best outline of cells and bacteria.

Examine under the oil-immersion lens for—

1. Type of Cell—Polymorphonuclear.
Monocyte.
Lymphocyte.
Epithelial cell.

2. Microörganisms—Morphology.
Frequency.
Intracellular or extracellular.

To classify bacteria when present use Gram's stain on Slide 2 as follows:

1. **Crystal violet, one minute and wash.**
2. **Gram's iodine, one minute and wash.**
3. **Alcohol or acetone to decolorize.**
4. **Safranin twenty seconds.**

Gram-positive is purple. Gram-negative is red.

Gram-positive Bacilli.

Diphtheria. Bacillus Welchii.
Tubercle. Malignant edema.
Boas-Oppler. Tetanus.
Subtilis. Anthrax group.
All other bacilli are Gram-negative.

Gram-negative Cocci.

Gonococcus.
Meningococcus.
Micrococcus catarrhalis.
All other cocci are Gram-positive.

LABORATORY MEDICINE–NICHOLSON.

AN OUTLINE OF THE ROUTINE EXAMINATION OF URINE.

The urine should be fresh. The following order of examination is the most time-saving:

1. If there are more than one, number all specimens and containers.

2. Mix well and centrifuge a test-tube full for at least four minutes.

3. **Sugar Test.**—To 5 cc. of Benedict's solution (about 1 inch in a test-tube of $\frac{3}{4}$ inch diameter) add 0.5 cc. (8 drops, not more) of urine. Heat over a flame for one to two minutes, shaking the tube to prevent solution spurting out, or better, stand the tube in boiling water two to three minutes, not more!

Negative—Clear blue or bluish-green with gray precipitate.

Positive—Opaque with a yellow or red sediment. The amount of sediment depends upon the percentage of sugar present. If the result is doubtful, allow to cool spontaneously before reading. If the sugar test is positive, test for ketone bodies by the nitroprusside reaction.

4. **Albumin Test.**—Fill a $\frac{1}{2}$ inch test-tube to the 2.5-cc. mark with clear urine and to the 10-cc. mark with 3 per cent sulphosalicylic acid. Mix. A white turbidity indicates albumin. Small amounts produce an opalescence, large amounts an opaque cloud. For quantitative estimation compare with standards.

5. Record, (a) hour passed.

(b) Color—dark yellow, light yellow, watery ,etc.

(c) Clearness or turbidity.

(d) Reaction, use blue litmus. Acid renders it red; alkali, blue.

(e) Specific Gravity—see that the urinometer floats freely. Make correction if at body temperature.

LABORATORY MEDICINE—NICHOLSON.

MICROSCOPIC EXAMINATION OF THE URINE.

Examine a well-mixed drop of sediment under a cover-glass, using the low-power lens with subdued light. Look for—

(a) Pus cells.
(b) Red blood cells.
(c) Casts.

Disregard cylindroids, mucus, epithelial cells, bacteria, crystals, amorphous sediment and extraneous matter. Less than 50 cells per low-power field or less than 3 perfect finger-shaped casts in the whole sediment is of no significance.

Cells.—If more than 50 cells are present under low-power, differentiate the pus cells from the red blood cells, under the high-power lens reporting the approximate number per high-power field after examining 10 microscopic fields and noting if the pus cells are clumped. Record over 100 cells as "much pus or blood."

Casts.—Use the low-power lens and if more than 3 typical casts are present state the approximate number seen in the whole sediment, reporting over 100 as "numerous." They do not have tapering ends or tails as do cylindroids. When more than one-half the cast is clear it is reported as hyaline; when more than one-half is granular it is reported as granular. Pus casts, blood casts or epithelial casts are reported as such. The high-power lens may be necessary to distinguish the cells on them.

7. Record findings.

LABORATORY MEDICINE—NICHOLSON.

EXAMINATION OF GASTRIC CONTENTS.

Directions to Patient.—A regular evening meal with 12 raisins at 8 P.M. No food or drink on the morning of the examination.

Pass the gastric tube about 20 inches. Withdraw the contents. Remove the tube.

Test-meal.—Eight arrowroot biscuits, 400 cc. of water.

In one to one and a half hours insert the tube and withdraw the contents.

Examine each specimen for amount, color and consistency (mucus).

Acidity.—By dipping indicator papers into contents and noting pH. Normal—fasting pH 2 to 1.5, one hour after meal pH 1.7 to 1.3.

Or titrate for free HCl and total acidity.

Free HCl.—One cubic centimeter of filtered contents, add 1 drop dimethyl indicator. Titrate with tenth-normal NaOH until red changes to yellow. Multiply by 100. Normal—fasting 10 to 30, one hour after a meal 20 to 60.

Total Acidity.—One cubic centimeter filtered contents, add 1 drop of phthalein indicator. Titrate with tenth-normal NaOH to faint pink color. Multiply by 100.

Lactic Acid.—(If no free HCl is present.)—Add 2 drops of 5 per cent ferric chloride to $\frac{1}{2}$ inch of filtered contents. Add 6 drops of 8 per cent mercuric chloride solution. A deep yellow color indicates lactic acid. Test a tube containing distilled water as a control.

LABORATORY MEDICINE—NICHOLSON.

TESTS ON FECES.

Naked Eye.—Note form of stool, presence of blood, pus or mucus.

Occult Blood.—Dissolve a benzidine powder in 5 cc. of 50 per cent acetic acid. Smear a central portion of the feces out on paper. Let fall on the surface a drop of the reagent. Green or blue in less than one minute indicates blood.

Parasite Eggs.—1. Shake the broken up feces in a quart-size fruit sealer containing water.

2. Allow sediment to settle three minutes. Pour out most of the water and add more, shaking as before.

3. Repeat two or three times to wash away bacteria. Remove large particles by filtering through coarse gauze.

4. After it has passed through the gauze and settled transfer a drop of the fine sediment to a slide and examine microscopically. Ova appear as many uniform bodies.

Bile.—Bile gives a red color when the stool is rubbed up with saturated mercuric chloride and allowed to stand several hours. Absent in obstruction of the bile-duct.

LABORATORY MEDICINE—NICHOLSON.

BLOOD UREA. (MERCURY COMBINING METHOD.)

1. To 7 cc. of 10 per cent trichloracetic acid in a graduated centrifuge tube add 7 cc. of oxalated blood, a few drops at a time, shaking between each addition.

2. Centrifuge at high speed for five minutes. Pipet off 5 cc. of clear supernatant fluid.

3. To this add 5 per cent bichloride of mercury until saturation occurs. Excess bichloride gives a faint brown color when added to saturated solution of sodium carbonate. 1.5 cc. bichloride may be used at once for the normal blood urea. If a drop of the mixture added to sodium carbonate on a white plate does not show brown in three seconds add 0.25 cc. of bichloride. Mix and test with sodium carbonate. Repeat until color appears.

4. Multiply cubic centimeters of 5 per cent bichloride used by 40 and subtract 60 to obtain milligrams of urea per 100 cc. of blood. Normal 20 to 35 mgm. (Urea $N_2 = \frac{1}{2}$ urea.)

BLOOD SUGAR (FOLIN-WU).

1. Add 2 cc. of oxalated blood to 14 cc. of distilled water. Add 2 cc. 10 per cent sodium tungstate. Shake. Add 2 cc. of two-thirds normal sulphuric acid, a few drops at a time shaking between each addition. The mixture should be chocolate-brown. Filter.

2. In a Folin sugar tube marked "unknown" place 2 cc. of filtrate. In another marked standard place 2 cc. standard containing 0.2 mgm. dextrose. To each tube add 2 cc. alkaline copper tartrate solution. Place tubes in boiling water six minutes. Then in cold water two to three minutes. To each tube add 2 cc. molybdate phosphate solution. Dilute both tubes to 25-cc. mark. Compare with colorimeter.

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 100 = \text{mgm. of glucose per 100 cc. of blood.}$$

Normal 80 to 120 mgm. per 100 cc. of blood.

LABORATORY MEDICINE—NICHOLSON.

COMMON LENGTH, WEIGHT, VOLUME AND TEMPERATURE EQUIVALENTS.

LENGTH EQUIVALENTS.

1 millimeter (mm.) = $\frac{1}{25}$ inch.
25 millimeters = 1 inch.
1 centimeter (cm.) = $\frac{2}{5}$ inch.
2.5 centimeters = 1 inch.
1 meter = 39 inches.

WEIGHT EQUIVALENTS.

1 milligram (mgm.) = $\frac{1}{64}$ grain (gr.).
1 gram (gm. or g.) = 15.5 grains.
4 grams = 1 dram (ʒ).
28 grams = 1 ounce (℥).
1 kilogram (kgm.) = 2.2 pounds (lb.).

VOLUME EQUIVALENTS.

1 cubic centimeter (cc.) = 16 minims. (m.).
4 cubic centimeters = 1 fluid dram (fl. ʒ). (approximately)
30 cubic centimeters = 1 fluid ounce (fl. ℥). (approximately)
473 cubic centimeters = 16 fluid ounces (U. S. pint).
568 cubic centimeters = 20 ounces. (Imperial pint).
1 liter = 34 fluid ounces.

EQUIVALENTS. Cent.	Fahr.	ACTION OF TEMPERATURES.
125 =	257	20 pounds steam complete sterilization in fifteen minutes.
100 =	212	Kills pyogenic microbes in one minute (not spores).
65 =	149	Kills pyogenic microbes in ten minutes.
55 =	131	Destroys complement in serum in fifteen minutes.
37 =	98	Body heat; best for growing microbes.
4 to 10 =	39 to 50.	Ice-box temperature best for preserving serums or vaccines.
0 =	32	Freezing.

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